

ENTOMOLOGY
(Medical and Veterinary)

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(MEDICAL AND VETERINARY)

BY

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P R E F A C E

On account of the war scarcity of books particularly of a technical nature has been experienced in India and with a view to easing the situation, the author undertook to write a book on medical entomology with the object of providing medical and veterinary students also public health workers with a treatise containing up-to-date information on the life history and bionomics of disease-carrying insects.

The information embodied in this book has been collected from various sources. A special effort has, however, been made to present the subject matter briefly without in any way affecting the essential details. It is not possible to provide keys which can be used for the identification of different insects. This would require a big volume by itself. However, taking into consideration the importance of *Anopheles* mosquitoes in the tropics, these have been exhaustively dealt with in this book. Keys and tables for the identification of adults and larvæ have been given. The key is mainly based on one salient character whereas in the table more than one distinctive feature has been given for a species. The beginner is advised to proceed with the identification with the help of the key, and where necessary, the identification thus made may be confirmed with the help of supplementary characters given in the table. The illustrations have not been drawn to any particular scale. As it is intended to bring out a short volume on insecticides and their practical application in medicine and public health, a separate chapter dealing with this very useful subject has been omitted.

The illustrations have been executed by Mr. J. K. Mullick, the artist, whose debt is acknowledged. The author is indebted for criticism, useful suggestions and other help to several colleagues among whom special mention should be made of Dr. S. M. Ghosh, his associate in the same department and Dr. N. Bhaduri, Research Worker in Helminthology. Dr. P. Bose of the Microbiology Department in the All-India Institute of Hygiene and Public Health was also kind enough to place at the author's disposal some specimens of mosquitoes and their larvæ from which drawings were made. Dr. A. G. McClymont deserves mention for help in the preparation of the manuscript for the press.

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CALCUTTA.

D. N. ROY.

The 25th January, 1946.

INTRODUCTION

Although it was known as early as 1869 that the embryos of guinea-worm are carried by water-fleas or cyclops, it was not till the discovery by Ronald Ross in 1898 of the relationship between malaria and mosquitoes that sufficient importance came to be attached to that aspect of entomology which is useful in preventive medicine. Entomology is really a branch of zoology but the medical man attaches the least importance to details of morphological or histological characters except those that have a direct bearing on the identification of the disease-producing agents. He pays special attention to their life history, bionomics, and natural enemies, his sole object being to discover a weak point in their life which will enable him to undertake the necessary measures to cope with the nuisance. Such measures should not only be effective but at the same time cheap, and easy to carry out.

It will thus be apparent that medical entomology has now a separate status which has its background in preventive medicine. The present war in the Far East has proved beyond doubt that entomology is the foundation of tropical medicine.

The word "Entomology" is derived from the Greek which signifies the science of insects. Strictly speaking therefore it should include the study of insects alone. Its scope has, however, been extended to comprise all animals in the phylum Arthropoda which in addition to insects also includes ticks, mites, tongue-worms, water-fleas, scorpions, centipedes etc.

On account of the presence of jointed appendages in at least one member of the phylum Annelida, there has been a tendency to include the two phyla, Annelida and Arthropoda, in a single phylum Appendiculata, and to give them the rank of subphyla. We will, however, consider them as separate phyla.

The animal kingdom is broadly divided into two sub-kingdoms, Protozoa or unicellular and Metazoa or multicellular animals. The latter is again subdivided into a large number of groups called phyla, and those of a lower order and which contain the important parasitic species are given below:

- (a) Platyhelminthes (tapeworms, flukes etc.)
- (b) Nematelminthes (roundworms, flukes etc.)
- (c) Mollusca (snails)
- (d) Annelida (earthworms and leeches)
- (e) Arthropoda (insects, ticks, mites etc.)

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PHYLUM ANNELIDA

The body is elongated and divided by a number of rings; the body cavity is a true coelom being lined by layers of mesoderm.

It comprises three different types of worms.

1. OLIGOCHAETA (earthworms): the locomotor appendages consist of chitinous setae or bristles attached in rows to the sides and ventral surface.

2. POLYCHAETA (bristle worms): the parapodia are highly developed bearing numerous long setae. There is a definite head with eyes and tentacles.

3. HIRUDINEA (leeches): possess locomotor and adhesive suckers either at both extremities or posteriorly; bristles on the body are absent.

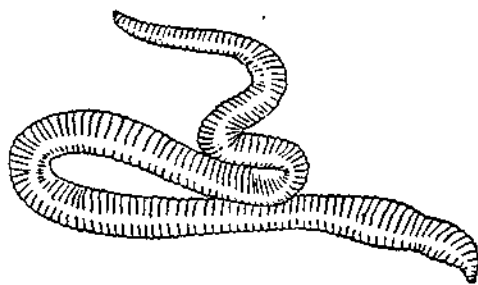


FIG. 1.
An earthworm.

OLIGOCHAETA or earthworms: These have been included by Blanchard among the pseudoparasites of man. Heymons (1926) and Müller (1926) have reported freshwater oligochaeta being passed in living states by patients. The species involved was *Pachydrilus lineatus*.

POLYCHAETA or bristle worms: Though the bristle worms are essentially marine animals they are very adaptable to changes in their environment, such as the salinity of water in which they may find themselves. There are only two instances where their occurrence in the human body has been reported. In the first case a specimen of the genus *Nereis* was evacuated from the nasopharynx of an adult individual who complained of coryza and severe headache. (Biswas and Strickland, 1927). The second case was reported by Strickland and Roy, (1933) where the specimen belonged to *Nereis* (*Lycoris*) *verille*, Grube, a typical marine species, which was passed in the stool of a child about 2 years old.

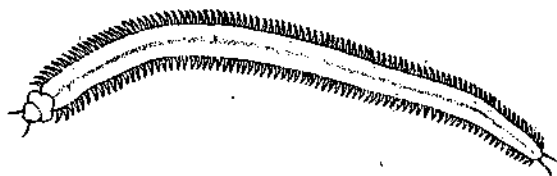


FIG. 2.
A polychaete (after Strickland and Biswas).

HIRUDINEA or leeches: The common leeches which concern us are (a) *Hamadipsa zeylanica*; (b) *H. sylvestris*; (c) *Limnatis nilotica*; (d) *Dinobdella ferox*; (e) *Hirudo medicinalis* and (f) *H. birmanica*. Their distribution and habitat are as follows.

H. zeylanica Moq-Tand.

This leech has a wide distribution throughout south eastern continental Asia and the Oriental islands. It is the only land-leech in Ceylon and is not found on the Indian mainland. They are extremely small and wiry looking. They are an intolerable pest in Ceylon attacking man and domestic animals.



FIG. 3.
Diagram of a leech.

H. sylvestris Blanchard.

In the living state they are much thinner than they appear in the preserved condition. This is the principal land-leech in Assam, Darjeeling, Sikkim and Burma.

A land-leech bites, and drops down on the ground after it has fed. It crawls up from the foot or shoes. It shows no colour preference. Its olfactory organs are poorly developed; it is not roused to activity by vibration. It can see objects clearly at a distance of 12 feet, and by sight alone it discerns its prey. This is the reason why people in the rear of a party going along a track through leech infested country are generally attacked.

Human beings and cattle are its favourite hosts; it shows a distaste for sheep and goats. It abounds in places where cattle graze; where cattle are absent, it is rarely found.

H. medicinalis Lin.

It is the common medicinal leech of Europe. In India this species is represented by *H. birmanica* which are found in tanks. They attack cattle.

L. nilotica Savig.

They are about 3 times as strong as the land-leech and are distributed in Palestine and Persia. They live in springs and similar water courses. They are known as "horse-leeches" on account of their habit of entering the air passages of horses and other domestic animals. The young leech is swallowed with drinking water and usually fastens on the mucous membrane of the mouth, pharynx, or larynx. In this situation it may even cause death of the host. Man is occasionally attacked; in the last Gallipoli campaign several cases occurred among soldiers.

D. ferox Blanchard.

These are of very large size and are found in tanks, ponds and in marshes. They are essentially cattle leeches and the report of the oozing of blood from the nostrils of domestic animals refers to an invasion of the air passages by this species in India. The parasitic infestation is generally of long duration.

This species is widely distributed in India, Burma and Ceylon. In the dry zones and also in the deserts they are conspicuously absent.

H. birmanica Blanchard.

It is the common medicinal leech of India. It is found in rivers, streams, swamps, tanks or ponds. It is widely distributed in India.

ANTI-LEECH REMEDIES.

The following observations have been made by Roy and Ghosh:

Infusion of tobacco leaves, copper sulphate, and common salt are no doubt toxic to leeches but they do not possess any repellent properties. In this respect nearly all volatile oils such as oil of citronella, lemon grass oil, oil cassia, oil ocimum, are not only toxic to leeches when applied directly on their bodies, but they also possess some repellent properties. Of these oils, those of cassia and citronella seem to be the most powerful either when prepared with vaseline, a drachm to an ounce, and applied on the skin, or mixed with rectified spirit in the same proportion as above and sprayed on socks. When the treated parts are washed with water, the repellent action will still persist for at least six hours. If applied directly to the leather of shoes or boots the repellent is less effective. The spirit preparation should be sprayed with a De Vilbiss sprayer on the socks around the ankle about $2\frac{1}{2}$ inches above and $\frac{1}{2}$ to $\frac{3}{4}$ inch below and also opposite the eyelets of shoes; complete protection against their bite for the whole day is thereby ensured.

Putties when wetted with infusion of tobacco leaves do not repel leeches but when they have crawled and stayed on the wet parts for about a minute or so, the animals are stupefied and are soon killed or they drop down in a semi-dead condition. Washing nut soaked in water is useful so long as it is wet, but as soon as the soap dries, it ceases to act.

TREATMENT OF INFESTATION OF THE NASOPHARYNX.

For the treatment of infestation of the nasopharynx *aqua aurantii floris* is ideal. When the official preparation (40 per cent strength) is diluted with an equal volume of water and sprayed by means of a De Vilbiss sprayer into the nasopharynx through the nostril in the case of cattle, and preferably through the mouth in the case of human subjects, it at once acts on the leech and causes it to become dislodged. This substance is extremely toxic to leeches. When *aqua aurantii floris* is not available, Eau De Cologne, which contains it, can be used. It is harmless to the mucous membrane.

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PHYLUM ARTHROPODA

The word Arthropoda has been derived from the Greek which signifies animals which possess jointed appendages. Entomology is no longer restricted to insects alone but it includes the study of the whole phylum Arthropoda. Insects form not only the largest group among this phylum but also the largest group of the animal kingdom. At least a quarter of a million different species have already been described. Besides being the largest, they are undoubtedly the most important group of arthropods. Other members of this phylum such as spiders, ticks, mites and centipedes also play some rôle in the well-being of man.

Although the presence of jointed appendages is an important feature of this phylum, it cannot be held as an axiomatic truth that all jointed limbed creatures must belong to this group. The chief characters distinguishing this phylum from others are:

- (a) Presence of metameric segmentation of the body.
- (b) Presence of bilateral symmetry of the body.

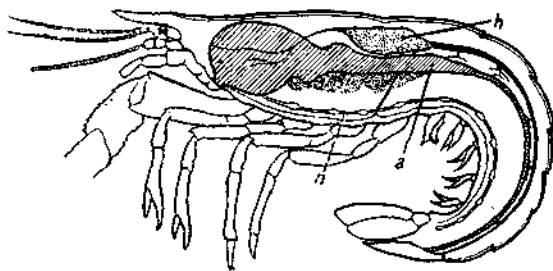


FIG. 4.
Side view of a prawn showing the relative positions of the alimentary canal, heart and nerve chain.

a, alimentary canal; h, heart; n, nerve chain.

(c) Central nervous system lies ventrally, the heart dorsally, and the alimentary canal between them.

(d) Each typical segment bears a pair of jointed appendages.

(e) Muscles are of the striped kind.

(f) Body cavity corresponding to hæmocoele which is in free communication with the circulatory system.

ARTHROPODA IN RELATION TO DISEASES AND DISCOMFORTS OF MAN.

- (1) They are obnoxious pests, *e.g.*, house-flies, eye-flies etc.
- (2) They bite, producing at times a certain amount of unpleasant after effects, *e.g.*, mosquitoes, fleas, ticks, lice etc.
- (3) They produce a kind of diseased condition by their presence on the skin, in one of the natural orifices of the body, or in the intestine, *e.g.*, external and internal myiasis, scarabiasis or beetle infestation, etc.
- (4) They produce dermatitis by their irritating effects, *e.g.*, spider-lick dermatitis, caterpillar hairs causing conjunctivitis, also dermatitis, etc.
- (5) They produce symptoms of local and general toxæmia due to the introduction of a venomous glandular secretion, *e.g.*, centipede, spider, scorpion, wasp, etc.

(6) They carry disease germs mechanically, *e.g.*, house-flies and cholera, abanid flies and trypanosome of surra, blood-sucking flies and anthrax, etc.

(7) They act as vectors or true intermediate hosts in which the development of the pathogenic organisms always takes place, *e.g.*, mosquitoes and plasmodial parasites of man and birds, mosquitoes and filaria, sandflies and leishmaniasis, tsetse fly and sleeping sickness etc.

HOW ARTHROPODA CAUSE HARM TO ANIMALS.

(1) Loss of blood causing reduction of the yield of milk and deterioration of health.

(2) Mechanical carriers of disease germs of anthrax, surra, etc.

(3) True intermediate hosts of disease organisms, *e.g.*, ticks causing piroplasmosis, spirochaetosis, etc.

(4) Flies of the family Oestridae cause considerable damage to hides and to meat.

(5) Paralysis and death may be caused due to some toxic action.

CLASSIFICATION:

In order to understand the systematic position of the different members in the animal kingdom, it is necessary to classify them into different groups and sub-groups, proceeding from the higher gradually to the lower grades. This classification is based generally on their external characters, *e.g.*, segmentation of the body, presence of wings; in addition to these, metamorphosis and habitat are also taken into consideration. The following table represents an outline of the different grades into which Arthropoda is subdivided. Only those which are important to us have been included.

Phylum Arthropoda (jointed-limbed animals).

Class Myriapoda
(Centipedes & millipedes).

Class Crustacea
(Prawns, crabs, cyclops).

Class Arachnida
(Ticks, mites, etc.).

Class Hexapoda
(Insects).

Each class may be subdivided into subclasses, orders, suborders, families, subfamilies, genera and species. To illustrate this point, we take a mosquito.

Class—Hexapoda. Order—Diptera. Suborder—Orthorrapha.

Family—Culicidae. Subfamily—Culicinae. Genus—*Culex*. Species—*fatigans*.

All family names end in -idae; subfamily names similarly end in -inae.

Each species represents both the genus and the species, *e.g.*, *Culex fatigans* in which *Culex* is the generic name and *fatigans* the specific name.

The generic name always starts with a capital letter and both are underlined.

THERAPEUTIC USES OF ARTHROPODA AND THEIR PRODUCTS.

(1) Use of malaria-infected mosquitoes for inducing malaria in cases of paresis.

(2) The treatment of arthritis with bee venom.

(3) The application of extracts from certain Meloid beetles as counter irritants.

(4) The use of blow-fly larvæ or extracts from them in the treatment of infected wounds.

(5) Leeches are employed for letting out blood.

(6) Hirudin has well-marked physiological properties.

Class Insecta

(Hexapoda)

The study of insects from an economical point of view is very important. It is seldom realised that all insects cannot be styled as pests, as some of them are no doubt beneficial to man. The part they play in the pollination of plants is the least appreciated by the ordinary man. Honey, silk, wax and cochineal dye are the products of insects. On the other hand, the well-being of man is entirely at the mercy of insects. They not only carry human and animal diseases, but also cause considerable harm to crops, plants and trees.

The Class Insecta is divided into many orders, the important among them being the following:

- (1) Diptera (Mosquitoes and flies).
- (2) Siphonaptera (Fleas).
- (3) Hymenoptera (Ants, bees, wasps etc.).
- (4) Lepidoptera (Butterflies and moths).
- (5) Coleoptera (Beetles).
- (6) Anoplura (Sucking lice).
- (7) Mallophaga (Biting lice).
- (8) Rhynchota or Hemiptera (Bugs).
- (9) Orthoptera (Cockroach, locusts etc.).

The chief characters of Class Insecta are: (1) Body regionally divided into head, thorax and abdomen; (2) Head carries a pair of jointed antennæ; (3) Mouth parts are adapted either for chewing hard food or for sucking only liquid food; some have their mouth adapted for piercing the skin and for sucking blood; (4) Metamorphosis may be either complete or incomplete; (5) Sexes are separate; (6) Thorax carries 3 pairs of legs and 2 pairs of wings.

TYPE: COCKROACH

(*Blatta orientalis* or *Periplaneta americana*)

Blatta orientalis: The pronotum is uniformly coloured without any dark markings.

Periplaneta americana: The pronotum has a faint yellowish border.

EXTERNAL STRUCTURE.

Head: The head does not show any indication of segmentation but is really composed of a pre-antennary and five further segments, ultimately united together.

The head can be separated into the following regions:

- (a) Epicranium covers the dorsal and posterior surfaces of the head.
- (b) Clypeus is a broad plate covering the front of the head below the epicranium.
- (c) Labrum, which is the upper lip.
- (d) Genæ or cheeks covering the sides of the head, behind and below the eyes.
- (e) Eyes are reniform elevations one on each side of the head. They are compound, being made up of numerous ommatidia.

The head bears: (1) A pair of many-jointed flagella-like antennæ. To the inner sides of the bases of the antennæ are a pair of small white oval patches called the fenestra and which represent a highly primitive ocellus.

(2) Mouth organs: (a) Labrum or upper lip overhanging the aperture of the mouth. (b) A pair of stout mandibles which lie below the genæ and articulate with the sides both of the epicranium and of the clypeus; their inner edges are toothed. (c) Behind the mandibles are the first pair of maxillæ (jaws). Each maxilla consists of two segments, cardo and stipes, the latter supporting an outer lobe, the galea and an inner lobe, the lacinia. To the maxilla is attached a 5-jointed palp. While with the help of the powerful mandibles solid food is chewed, the maxillæ assist in holding and masticating the food. (d) Behind the first maxillæ are the second maxillæ which have the two basal segments united together into a single structure which supports the prementum; the latter carries two pairs of distal lobes, an outer pair, paraglossæ, and an inner pair, the glossæ; these two structures collectively form the ligula. The united 2nd maxilla is called the labium or lower lip. A pair of 3-jointed labial palps is attached to the prementum. A soft median process, the tongue (lingua or hypopharynx) is attached to the upper surface of the mentum.

Thorax: The thorax consists of three separate segments, prothorax, mesothorax and metathorax. The dorsal plate of each segment is called the tergum, the ventral plate is the sternum, and the plate which joins the tergum and the sternum laterally on each side is the pleuron.

Attached to the anterior border of the tergum of the mesothorax in the male are the anterior wings or elytra. These are highly chitinated and hard. They take no part in the flight of the insect and their function is merely to protect the more delicate second pair of wings which are membranous. When at rest they are kept folded up longitudinally like a fan under the elytra. The second pair of wings articulate with the tergum of the metathorax. In the female of *Blatta orientalis* the wings are vestigial.

To the ventral surface of each segment of the thorax is attached a pair of legs. On account of the presence of three pairs of legs, insects are often called hexapoda. Each leg is composed of: (1) coxa, (2) trochanter, (3) femur, (4) tibia, (5) 5 tarsal joints; to the last segment are attached the pulvilli and a pair of claws.

Abdomen: The abdomen consists of 10 segments. Each segment is enclosed by a dorsal tergum and a ventral sternum. In the male the 9th segment bears a kind of rudimentary styles. In the female the sternum of the 7th segment is very much more prominent than in the male. The anus lies below the 10th tergum which is produced backwards into a thin flexible plate, notched at the posterior border.

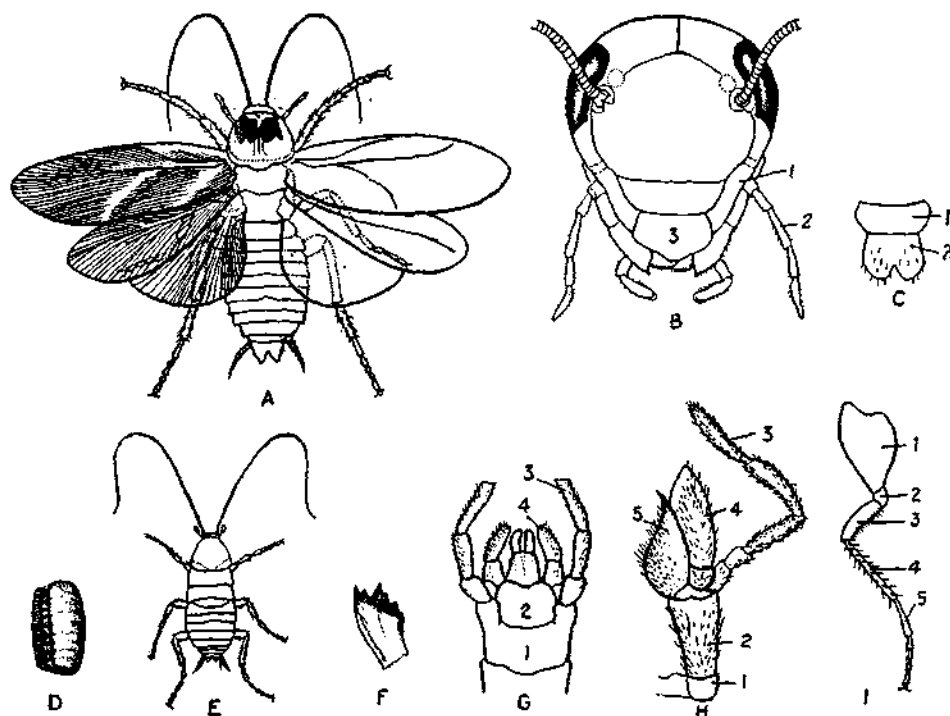


FIG. 5.

- A. Diagram of a cockroach (Class Insecta).
 B. Front view of head of cockroach. 1. gena; 2. maxillary palp; 3. labrum.
 C. 1. clypeus; 2. labrum.
 D. Egg capsule of cockroach.
 E. Larva.
 F. Mandible.
 G. 1. submentum; 2. mentum; 3. labial palp; 4. paraglossa.
 H. 1. cardo; 2. stipes; 3. maxillary palp; 4. galea; 5. lacinia.
 I. Leg of cockroach. 1. coxa; 2. trochanter; 3. femur; 4. tibia; 5. first tarsal segment.

INTERNAL ANATOMY.

Digestive system: The digestive system consists of an alimentary canal which is divided into foregut, midgut, and hindgut. The foregut consists of (a) mouth; (b) buccal cavity which receives the duct of the salivary glands. Each gland is divided into two lobes. There is a separate salivary receptacle. The duct of the salivary receptacle joins with that of the salivary glands into a common duct and the two common ducts of each side unite to form a single duct; (c) pharynx which is provided with powerful muscles; (d) oesophagus; (e) crop; (f) proventriculus which is lined inside with teeth. The midgut consists of the stomach proper. To it are attached the hepatic cæca. The stomach with the hepatic cæca is the mesenteron; the regions in front and behind the cæca are the stomodæum and the proctodæum respectively, the two last-named portions being lined by a chitinous cuticle.

The point of junction between the stomach and the intestine is indicated

by the attachment of the Malpighian tubules which are excretory in function. The Malpighian tubules are yellowish, thread-like, and extremely long.

For the digestion of the particular types of food, corresponding enzymes are present in the salivary glands and stomach, according to feeding habits of the insects.

Circulatory system: The fluid blood circulates in the hæmocœle and does not flow in any well-defined tubular structure except in the heart and aorta. The heart is an elongated tube, closed behind and open in front, running along the middle line of the abdomen and thorax immediately behind the terga. The aorta is a narrow tube which is a continuation of the heart anteriorly.

The heart is divided by valves which open forwards into a number of chambers; its walls are provided with valvular apertures or ostia through which blood enters the heart. The blood is driven forwards by rhythmical contractions.

The blood plasma is called the hæmolymph. It is composed of protein, lutein, metallic salts, and also food absorbed from the midgut, and some cellular elements. It also contains some enzymes.

Respiration: Respiration takes place through tracheæ which ramify over every organ of the body. They possess a chitinous internal lining. They open on the surface at the stigmata through which the air enters the trachea. One pair of stigmata lies on the side of the thorax between the prothorax and mesothorax and the second pair between the mesothorax and metathorax. Eight are situated on either side of the abdomen between the terga and sterna of the segments. Each spiracle is guarded by a valve.

Air enters the body by diffusion through the wall of the trachea, part of the CO₂ being eliminated through the cuticle. The tracheoles come into play when an excess of oxygen is needed for extra work performed by muscles, e.g., during flying and running. In some animals which have no tracheal system, the air enters through the cuticle which acts as a membrane.

Excretion: Solid excreta are given off in crystalline form or in aqueous solution in which the Malpighian tubules play the most important part. Nephrocytes which are special cells arranged in groups are supposed to possess excretory function as after injection of ammonia carmine into the blood, carmine particles are retained in the cytoplasm of these cells.

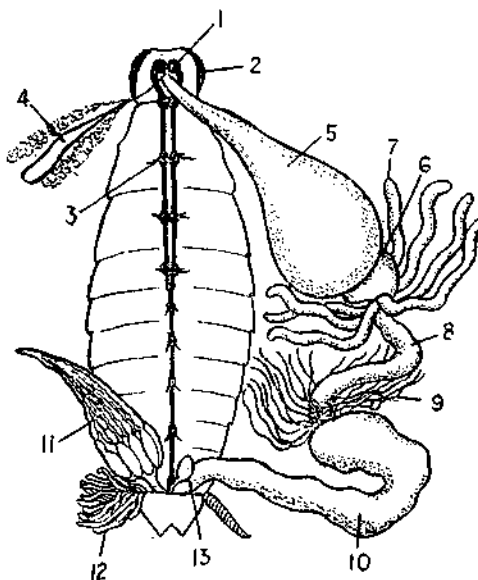


FIG. 6.

Dissection of female cockroach.

1. cerebral ganglion; 2. eye; 3. ventral nerve chain with ganglia; 4. salivary glands with salivary vesicles; 5. crop; 6. gizzard; 7. hepatic caeca; 8. midgut; 9. malpighian tubules; 10. hindgut; 11. ovary; 12. colleterial glands; 13. spermatheca.

The proximal regions of the Malpighian tubules are supplied with systems of circular and longitudinal muscles external to the basement membrane. The muscles are continuous with those of the midgut. Eastham (1925) has observed peristalsis occurring in the tubes and in this respect he does not agree with Keilin (1921) who holds that these tubes do not exhibit any peristaltic movement.

REPRODUCTIVE ORGANS.

Male: (a) a pair of testes with corresponding vasa deferentia; (b) a pair of vesiculæ seminales, two tufts of whitish cæca which together constitute what is known as the "mushroom-shaped gland"; these open into the anterior end of (c) the ejaculatory duct which opens between the 9th and 10th sterna.

The characteristic smell of the cockroach is due to large branched tubular glands in the male insects.

Female: (a) a pair of ovaries each consisting of 8 ovarioles; (b) the two oviducts unite into a single tube which opens in a median aperture on the 8th abdominal sternum; (c) a pair of receptaculum seminis or spermatheca which open into the common oviduct; (d) colleterial glands, a pair of ramifying glandular tubules which open behind the spermatheca, their calcareous secretion helping to form the egg-capsule.

THE STRUCTURE OF AN OMMATIDIUM.

An ommatidium is a component unit of a compound eye and consists of a complete visual element.

The outer layer is the cornea lying in a hexagonal facet and is often thickened to have the shape of a biconvex lens. Beneath the cornea is the corneagen; these cells form new cells for the cornea. The cornea of adjacent ommatidia form a continuous sheath. Beneath the corneagen is the crystalline cone which is enclosed in certain large cells. Below the cone lies a rod-like body called the rhabdom, which has a striated appearance and is formed from the secretion of the retinal cells. Pigment cells lie between the cone and the rhabdom of the separate ommatidia. The rhabdom is regarded as the perceptive part of the ommatidia. The rhabdom is separated from the nerve cells and fibres by a thin membrane.

The simple eye or ocellus is fundamentally the same as one of the visual elements of a compound eye. It has only one lens and each retinal unit is a single cell, of which the distal part is unpigmented.

A simple eye is capable of perceiving light and darkness but for more clear vision, e.g., for perception of motion, a compound eye is essential. There is no focussing mechanism in the eye of an insect.

LIFE HISTORY OF *Periplaneta americana* LIN.

The life history of this cockroach as worked out by Nigam (1933) at Pusa is given below.

This cockroach is ubiquitous and omnivorous, infesting storerooms, kitchens, etc. and attacking clothes, hair, boots, paper, books, fruits and particularly

sweetened and starchy foods. Both nymphs and adults are attracted to warmth, moisture, and darkness, and can live for several days without food. The eggs are enclosed in a bi-valve chitinous covering called an egg-pod. These are laid from April-May throughout the summer months but very rarely in Nov.-March. The number laid by a single female is usually 10-15; in nature it is probably much higher. The average interval between oviposition is 4-5 days when the females have ample opportunities for pairing. There are normally 16 eggs in a pod. The female matures 1-2 weeks after the last moult and usually begins to oviposit 3-7 days after pairing. The eggs hatch in 27-28 days. The metamorphosis is incomplete or gradual. After a varying number of moults the nymphs reach maturity in 10-21 months. When deprived of food, they will devour their own kind, and they have been recorded as preying on termites and bed-bugs.

According to Klein (1933), the preoviposition period is 10 days at 26-28°C. The formation of the egg-pods takes 1 day in summer. A pod contains an average of 15 eggs, and the average number laid by a female is 21.5 with a maximum of 46. The incubation period lasts 1-2 months. The nymphs moult 6 times and complete their development in 12-34 months. The adult is able to live for a long time without food and water. The adult life is about 12 months.

RELATION TO DISEASE.

Ants and other Hymenoptera often parasitise the egg-pod. Some Hymenoptera and scorpions prey on the cockroach.

DISEASE.

On account of the habits of cockroaches, there is no reason why they should not act as carriers of cholera. In fact Barber (1914) has produced evidence that they may harbour cholera vibrios in their intestines and these may appear in enormous numbers in insect faeces after they have been fed on infected materials. They have also been found to pass viable hookworm eggs after feeding on the stool of an infected person.

DESTRUCTION.

Prevention is more or less impossible. They hide in the day time in any dark place they may find and emerge at night to feed. Drains and privies, pipes, barrels, holes in walls, store houses, kitchens, and godowns are their favourite resting places.

They show remarkable tolerance for arsenic; therefore, for rapid destruction, fumigation with sulphur dioxide is perhaps the best method. Of late sodium fluoride has been much in use. It has been employed both as a contact and as a stomach poison. Starch paste containing 1, 2.5, 5 per cent of sodium fluoride causes on an average, death of the insects in 6, 5 and 3 days respectively (Griffiths and Tauber, 1943). When dusted over hiding places it causes the death of the cockroaches in a few hours. In order to enhance its action it should be mixed with finely powdered pyrethrum. Powdered borax mixed with sugar or starch is also an effective stomach poison. It may also be used mixed with either

honey or treacle. According to some, a mixture of borax (2 parts), salicylic acid (1 part) and sodium silico-fluoride is an effective poison bait for use indoors.

Pyrethrum spray causes the females to drop their egg capsules. Cockroaches affected by pyrethrum may remain in a moribund state even for 24 hours. Rotenone and pyrethrum are equally toxic to the cockroach provided the necessary quantity has been introduced into the body.

CLASSIFICATION

The above represents the characters of a cockroach which has been taken as a typical representative of the class Insecta. When a cockroach is compared with any other insect, a marked diversity in animal life is clearly recognised as a consequence of which no two insects are exactly alike. The differences may be either small or markedly conspicuous. At the back of such diversity lies the evolutionary changes principally brought about as a result of environment. It is for this reason that the mouth parts of a mosquito are adapted for piercing and sucking and those of a beetle for chewing. The loss of wings in a louse is also the result of adaptation to parasitic conditions. Such diversities in both the external and internal features of an insect together with its physiological behaviour have been largely employed in classifying them into different groups.

Insects are divided into a large number of orders on the presence or absence of wings, metamorphosis, and their outward structure, especially of the mouth parts.

Subclass I—Apterygota which are completely devoid of wings and pass through no metamorphosis. This group includes such orders as Thysanura ('silver-fish'), and Collembola (spring tails).

Subclass II—Pterygota which have wings in at least one of the two sexes in the adult state. Metamorphosis is either complete or incomplete. They have further been classified into:

Exopterygota.

(Heterometabola)

Pterygota which pass through a simple or slight metamorphosis; development of wings external.

Endopterygota.

(Holometabola)

Wings start as invaginations inside the body; metamorphosis complete.

Exopterygota or Heterometabola.

Order Orthoptera (cockroach, grass-hopper, etc.).

Order Dermaptera (ear-wig).

Order Plecoptera (stone-flies).

Order Ephemera (May-flies).

Order Odonata (dragon-flies).

Order Hemiptera (bugs).

Order Mallophaga (biting lice).

Order Anoplura (sucking lice).

Order Neuroptera (ant-lions).

Order Mecoptera (scorpion-flies).

Order Trichoptera (caddis-flies).

Endopterygota or Holometabola.

- Order Diptera (flies).
- Order Coleoptera (beetles).
- Order Hymenoptera (ants, wasps, etc.).
- Order Lepidoptera (butterflies and moths).
- Order Siphonaptera (fleas).

A. Diptera, Anoplura, and Siphonaptera are directly related with the transmission of important diseases of man and animals.

B. Neuroptera, Mecoptera, Trichoptera, Ephemera, Odonata, Plecoptera are important as their larvae prey on larvae of mosquitoes.

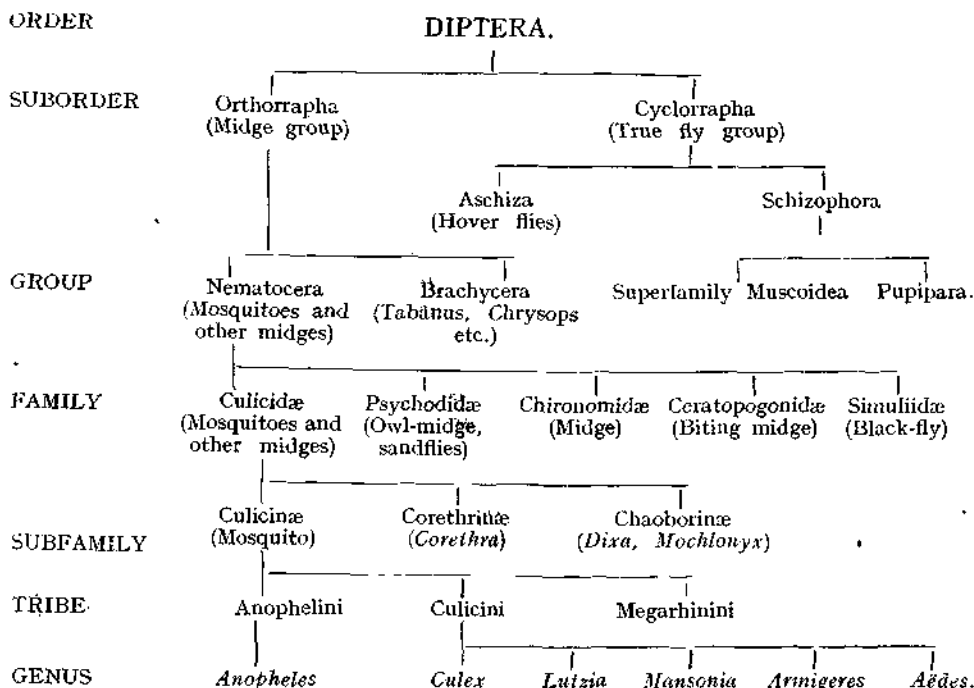
C. Coleoptera, Lepidoptera are destructive to stored food.

D. Coleoptera, Orthoptera, Lepidoptera, Diptera are destructive to plant life.

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DIPTERA.



The members of this order, which includes mosquitoes and flies, are of considerable importance to man, especially in the dissemination of diseases. The diseases transmitted by mosquitoes alone are responsible for as much loss of human lives as are those of all other pathogenic diseases.

The order Diptera possesses all the characters of the class Insecta except that the hind pair of wings is replaced by a pair of club-shaped organs used for balancing and called halteres. The metamorphosis in Diptera is complete. This order is divided into two suborders, Orthorrapha and Cyclorrapha. The former represents in addition to mosquitoes and midges other insects like *Tabanus*, *Chrysops* etc. Cyclorrapha includes the true flies. The difference between the two suborders is shown below:

Orthorrapha.

Represents the midge group of insects, also gad-flies. (a) Larval head is distinct. (b) The last larval skin is shed and the pupa is naked, i.e., it is not enclosed in the larval skin. (c) The adult escapes through a longitudinal slit that appears on the back of the cephalothorax of the pupa.

Cyclorrapha.

Represents flies, both biting and non-biting, also tick-flies. (a) The larva is maggot like and the head is not differentiated. (b) The last larval skin is not shed; it hardens to form the puparium which encloses the pupa. (c) The adult escapes from the puparium through a circular slit that appears at its anterior part.

Orthorrhapha is subdivided into two groups: (1) Nematocera, and (2) Brachycera, and their principal characters are given below:

Nematocera.

(Mosquito and other midges)

(a) Antennæ long and filamentous and composed of a large number of segments which are more or less similar in appearance.

(b) Maxillary palps are elongate and flexible and are commonly composed of 4 or 5 joints.

(c) The third longitudinal vein is never forked though several veins are branched.

Brachycera.

(Gad-flies)

(a) Antennæ much shorter and seldom filamentous; commonly composed of 3 dissimilar segments.

(b) The palps are not flexible and usually much shorter; composed of 2 segments.

(c) Several longitudinal veins may be forked but never the 2nd.

Cyclorrhapha, which includes the true flies, is classified into two groups, Aschiza and Schizophora.

Aschiza.

(Hover flies)

Frontal lunule indistinct and frontal suture absent.

Schizophora.

(True flies including tick-flies)

Frontal lunule distinct and frontal suture present.

Orthorrhapha—Nematocera may be grouped into numerous families but from our point of view only four need be considered. These are Culicidæ, Psychodidæ, Chironomidæ (in which is included Ceratopogonidæ) and Simuliidæ. They are all blood suckers and some of them are well known carriers of disease-producing organisms.

Culicidæ (Mosquitoes).	Psychodidæ (Sandflies).	Chironomidæ (Midges).	Ceratopogonidæ (Biting midges).	Simuliidæ (Black flies).
2nd longitudinal vein forked only once; body and wings may or may not be covered with scales.	2nd longitudinal vein forked twice; body and wings densely hairy.	Short proboscis; mouth parts adapted for sucking only. The first longitudinal vein stops a little beyond the middle of the wing; no scales on wings and body.	Like Chironomidæ. Mouth parts adapted for piercing the skin and for sucking blood.	Fly-like with short but formidable proboscis; antennæ small, many jointed; and project out like horns of an animal; no scales on wings and body.

The family Culicidæ is further subdivided into three subfamilies, Dixinæ, Chaoborinæ and Culicinæ, of which the first two are incapable of piercing the skin and hence are unimportant.

Dixinae.
Mouth parts short and unfit for piercing; no scales or hairs on the wings and body.

Chaoborinae.
Proboscis short and not adapted for piercing the skin; no scales on the wing-veins; presence of fringe-scales along the posterior border of the wing.

Culicinae.
Long, slender proboscis; mouth parts of some adapted for piercing and for sucking; scales present on the body, legs and wings.

Dixinae: This group is confined to one genus *Dixa*, the larvæ of which are aquatic. The body of the larva is bent so that the anterior and posterior ends almost meet. Pseudopods armed with hooks are present on the ventral surface of some of the mid-abdominal segments. There is a respiratory cup on the last segment. The larva rests at the surface of water.

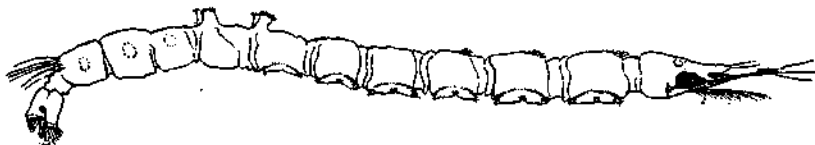


FIG. 7.
Larva of *Dixa* (after Miall).

Chaoborinae: The Chaoborinae form a small group of insects which closely resemble mosquitoes. The early stages are aquatic. The larvæ are predacious and feed on other insects or their larvæ. They are found in ponds along with Anopheline larvæ and like the latter they lie horizontally at the surface and often remain motionless for several minutes in which position they are almost invisible on account of their extreme transparency. They are devoid of mouth brushes, the antennæ being prehensile. The mandibles possess well developed teeth.

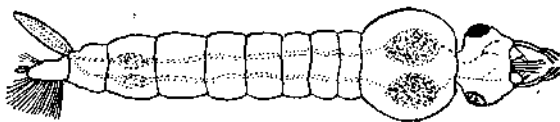


FIG. 8.
Larva of *Mochlonyx* (after Miall).

Culicinae: The Culicinae, which includes mosquitoes, can be easily differentiated from others by the following characters: (1) long, slender proboscis; (2) mouth parts of some adapted for piercing and sucking; (3) the presence of scales on wings and a fringe of scales along its posterior border; (4) characteristic wing venation.

The subfamily Culicinae is further grouped into tribes; only the following deserve our consideration:—

Tribe Anophelini

Tribe Culicini

Tribe Megarhinini.

Tribe Anophelini: Free edge of scutellum semilunar; the palpi are as long as the proboscis in both male and female; in the male the end of the palpi is clubbed; abdomen never uniformly invested by broad overlapping scales. They generally

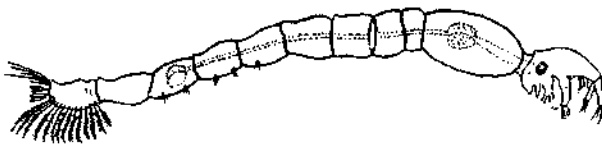


FIG. 9.
Larva of *Chaoborus* (after Miall).

rest with the back straight i.e. the head, thorax and abdomen being in a line ; some anophelines adopt the culicine attitude.

Tribe Culicini: Free edge of scutellum distinctly trilobed ; the palpi are short in the female and long in the male ; abdomen uniformly invested by close overlapping scales ; in the resting state they are hunch-backed.

Tribe Megarhinini: Free edge of scutellum indistinctly trilobed or semilunar ; proboscis distally bent like a hook ; abdomen completely invested with scales.

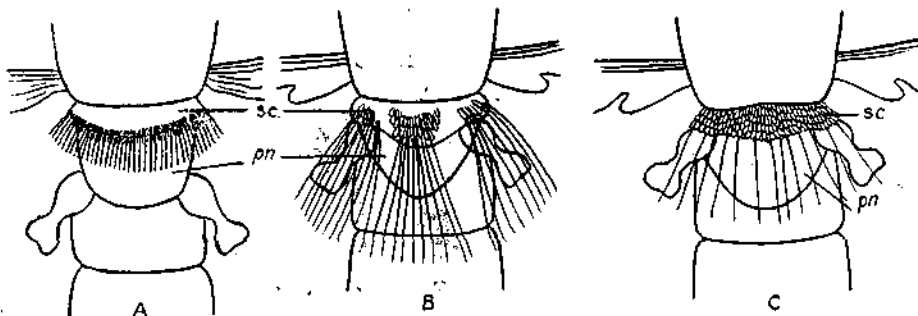


FIG. 10.
Mesoscutellum of (A) *Anopheles*, (B) *Culex* and (C) *Megarhinus*.
sc, scutellum ; pn, postnotum.

Tribe Megarhinini

Genus *Megarhinus*: The adult is of large size and is as a rule brightly coloured with green, blue, yellow, purple and white scales which have a bright metallic lustre. The proboscis is very long and has a characteristic shape. It thins out considerably towards the distal part and is bent like a hook. The insect is incapable of piercing the skin. In addition to the characteristic proboscis there are other characters which are helpful in the identification of this tribe. The posterior fork cell of the wing is very short ; there is a V-shaped thickening in the wing membrane between the branches of the 5th vein. The sexes are easily differentiated by the antennæ. The larvæ are carnivorous. Their mouth parts are structurally modified to enable them to catch hold on to living objects which come within their reach. Cannibalism is very freely observed among them. They feed on mosquito larvæ and other midges, even on tadpoles. Eggs are laid in clusters and larvæ are found in tree-holes, and bamboo stumps during the monsoon. These mosquitoes are all confined to the tropics.

***M. splendens*:** It is widely distributed in the tropics. Its chief characters are: abdomen with conspicuous tufts of hairs on segments 6, 7 and 8, those on 7 black, on 8 orange ; first abdominal tergite blue or green in the middle, pale yellow at the sides ; fore-tibia dark at apex.

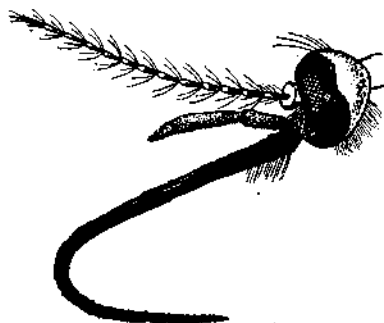
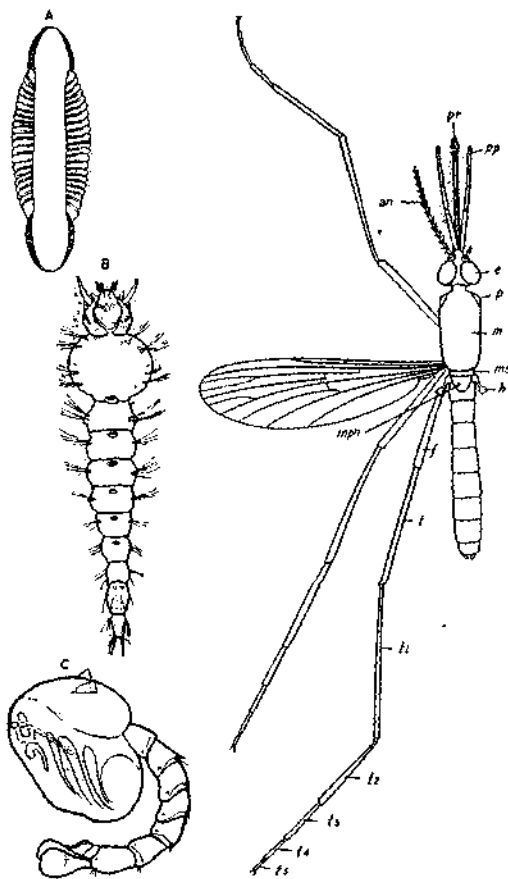


FIG. 11.
Head of *Megarhinus*.

The antennæ of the larva are almost bare and the hairs of the mouth brushes are peculiarly bent. The eggs are dropped on the surface of water from a height of 2 or 3 inches. The larvæ are carnivorous. Mating does not take place in captivity. The adults feed solely on the surface moisture of plants. On account of its peculiar habits of breeding in bamboo stumps, tree-holes, earthen pots etc., the larvæ of this mosquito have been used to suppress the breeding of *Aëd. scutellaris* in tree holes in Fiji with questionable results.

MOSQUITO (Subfam. Culicinae)
ADULT.

EXTERNAL CHARACTERS.



FIGS 12 & 13.

Showing the complete metamorphosis of a mosquito.

Fig. 12.—A. Anopheline egg. B. Anopheline larva. C. Anopheline pupa.

Fig. 13.—Diagram of a female *Anopheles*
an, antenna; e, eye; m, mesonotum; p, pronotum; pp, maxillary palp; pr, proboscis; f, femur; h, haltere; inpn, mesopostnotum; t1 to t5, tarsal

The body is divided into head, thorax and abdomen, a neck connecting the head with the thorax.

Head: Head is spherical and is produced anteriorly into a prominent clypeus.

The eyes occupy most of the head; behind them the latter is covered with scales. The eyes are compound, being composed of numerous ommatidia, each of which is a separate visual element. Ocelli are absent. Occiput is clothed with scales and hairs.

Each antenna is composed of 14 to 16 segments: the first antennal segment is small and is obscured by the large second segment or torus. The second is large and bulbous. On the second lies Johnston's organ, sensory in function. The third segment and successive segments are more or less alike. The antenna carries whorls of coarse hairs round the base of each segment, and numerous fine, short hairs are scattered over the rest of the surface. In the male the antenna is plumose, being densely hairy. In the female it is sparsely hairy or pilose. This affords a ready means of identifying the two sexes.

Proboscis: The proboscis is long and appears as a single organ projecting forward from the head. It consists of the labium which is long and slender, and at its distal end are two small triangular lobes the labellæ, said to represent the labial palpi. Between the two labellæ lies a fine membrane called Dutton's membrane. It was once thought that the microfilariae escaped from the proboscis of the mosquito by piercing this membrane and made their way into the wound. It is now known that the filariæ force their way through the tip of the labella.

The labium is clothed with scales, and is a flexible hollow structure with a longitudinal groove along the whole length of its dorsal surface in which the actual mouth organs lie concealed. These are (a) a pair of maxillæ, (b) a pair of mandibles, (c) labrum—epipharynx, and (d) hypopharynx.

The maxilla is thin and blade-like and is provided with a number of teeth at the distal end. The number of teeth varies in different species and it was once thought (Roubaud, 1921) that a definite correlation could be established between its number and the feeding habits of the mosquito in regard to its adaptability to pierce human or animal skin. The critical number of teeth fixed by him was 14, below which the females were human-feeders and above, animal-feeders. Toumanoff's results (1935) in Indo-China no doubt support the view held by Roubaud but this has not been corroborated by Senior White in India (1937). In males the maxillæ are reduced and the serrations are not so well developed.

Mandible: The mandible is much thinner than the maxilla; its distal end is very finely serrated. In the male the mandibles are very much reduced.

Labrum-epipharynx: In the mosquito the labrum and the epipharynx are fused to form a single structure.

Hypopharynx: The hypopharynx lies on the ventral surface of the labrum-epipharynx. It represents the tongue and arises from the floor of the mouth. It carries the salivary duct. At the time of feeding the lateral margins of the labrum-epipharynx appose with those of the hypopharynx and meet to form a complete tube for the passage of food. Vogel (1920) and Robinson (1939) are of the opinion that the food channel is formed by the labrum alone, the hypopharynx playing no essential part in its formation. As the salivary duct runs along inside the hypopharynx, the salivary secretion cannot come in contact with the contents of the food-tube except at the extreme distal end where the salivary duct opens.

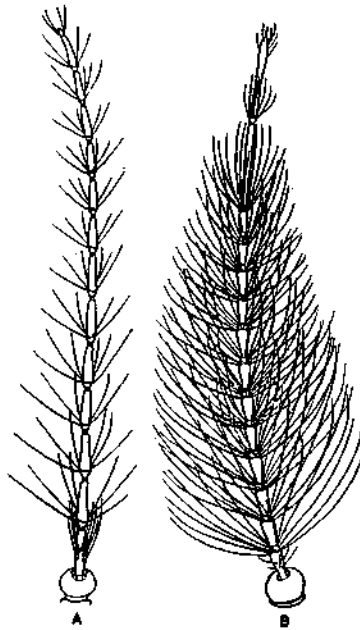


FIG. 14.
Antenna of mosquito: A, female; B, male.

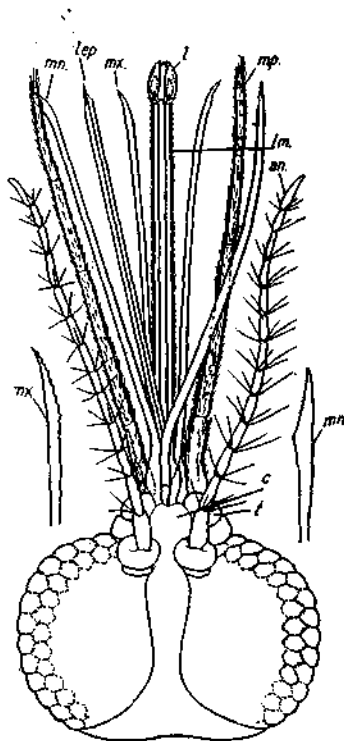


FIG. 15.

Head of a female *Anopheles* showing the different mouth parts. an, antenna; c, clypeus; l, labella; lm, labium; mn, mandible; mp, maxillary palp; mx, maxilla; t, torus or 2nd antennal segment; lep, labrum-epipharynx.

Thorax: The thorax is covered with scales and is formed by the fusion of the three segments, prothorax, mesothorax and metathorax. The wings are attached to the mesothorax and the halteres to the metathorax while each segment of the thorax bears a pair of legs.

Though the thorax is composed of three segments, the middle one or the mesothorax is the largest and occupies practically the whole of the dorsum of the thorax, the other segments being not at all prominent.

The prothoracic lobes lie at the antero-lateral regions of the mesothorax and project on either side of the neck. The pronotum is divided into two parts which are called anterior and posterior pronotal lobes. The coxa of the first pair of legs is attached to the prosternum.

MECHANISM OF THE BITE.

The female mosquito settles on the skin and with its proboscis feels for a suitable spot for puncturing the skin for biting. The labellæ are applied to the skin and as soon as they are pressed, it causes the labium to bend like an elbow and simultaneously the other mouth organs such as the mandibles, maxillæ, epipharynx and hypopharynx are protruded from the end of the labella on to the skin. The skin is punctured by the maxillæ and mandibles. The maxillæ probably enlarge the wound through which the epipharynx and the hypopharynx are pushed.

Palp.

The palpi are attached to the base of the maxilla. They show a great diversity of form and exhibit considerable difference in shape, and length. The difference in the two sexes is often very marked. The palpi are clothed with scales. The number of joints is variable but as a rule they are composed of 4 segments. When the living mosquito is at rest, the palpi are closely applied to the labium.

The vertex and the occiput are clothed with numerous scales. The palpi are clothed with scales except on the inner surfaces. Scales may be dark or pale.

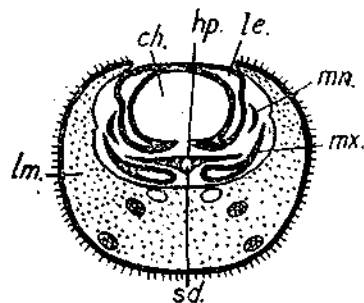


FIG. 16.

Transverse section through the proboscis of a female mosquito. (After Nuttall and Shipley).

ch, food channel; hp, hypopharynx; le, labrum-epipharynx; lm, labium; mn, mandible; mx, maxilla; sd, salivary duct.

The dorsal part of the mesothorax is domeshaped. The mesonotum or the sclerite which covers the mesothorax dorsally is divided by an incomplete suture, the mesonotal suture. This begins at the side in front of the anterior spiracle at a short distance from the anterior end of the thorax and runs upwards and backwards. The mesonotum is thus divided into an anterior part called mesoprescutum and a posterior one called mesoscutum. There is a conspicuous transverse ridge at the posterior part of the mesoscutum which separates the mesoscutum from the mesoscutellum. The shape of the posterior margin of the scutellum is important in the identification of the tribe. It is evenly rounded in Anophelini, distinctly trilobed in Culicini, and indistinctly trilobed or even semi-lunar in Megarhinini. The scutellum overhangs a dome-shaped plate which is known as the mesopostnotum.

The mesopleuron which forms the lateral wall of the mesothorax consists of two plates, mesoepisternum and mesoepimeron. The coxæ of the second pair of legs are attached to the mesoepisternum.

The metathorax is much reduced. The metatergum is present as a narrow bridge between the mesoscutellum and the first abdominal tergum. The coxæ of the third pair of legs are attached to the metathorax.

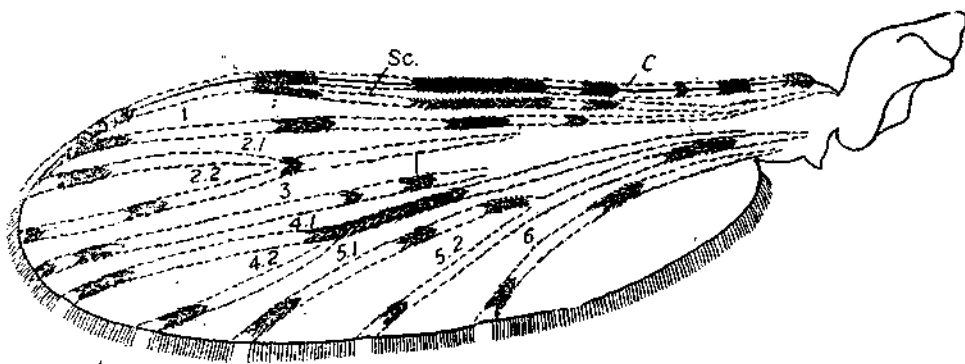


FIG. 17.

Wing of an anopheline mosquito.

C, Costal vein; Sc, Subcostal vein; 1-6, longitudinal veins.

The thorax bears two pairs of spiracles. The anterior pair lie on the mesothorax just posterior to the posterior pronotal lobe. The posterior pair lie on the metathorax above the insertion of the coxa of the third pair of legs. The spiracles are slit-like.

Halteres: They resemble drumsticks and represent the second pair of wings of insects. They are attached to the sides of the metathorax. They have been assigned different functions by different authors; these are sound production, maintenance of equilibrium or balancing, and the third, hearing.

Wings: The wing is attached to the mesothorax. It has a distinct shape and a characteristic venation. All wing veins are clothed with scales. The ornamentation or dappling of wings is entirely due to the arrangement of pale or dark scales on the veins.

On the wings there are eight veins, namely, the costa, subcosta, and six

longitudinal veins, the second, fourth, and fifth of which are forked. In addition, the posterior border of the wing is fringed with scales.

The costa, subcosta and the first longitudinal vein arise independently from the base of the wing. The fourth, fifth and sixth longitudinal veins arise together in a rather curious manner from a common cord-like structure. The second longitudinal vein originates at about the junction of the inner with the middle third of the wing. The third longitudinal vein is the shortest and runs to the apex of the wing.

A cross vein connects the third with the second and fourth longitudinal veins ; another cross vein is linked with the stem of the fourth and the upper branch of the fifth.

When the imago emerges from the pupa the wings are small, but they are soon distended and after a short interval, the upper and lower surfaces coalesce, leaving the tracheæ as veins.

Leg : The leg is thickly covered with scales. Each segment of the thorax, bears a pair of legs. Each leg consists of (a) coxa (b) trochanter (c) femur (d) tibia and (e) five tarsal segments, the last one ending in a pair of claws. The claws may be either simple or may contain one or two teeth. A considerable difference is noticed in the arrangement of these teeth not only in the two sexes but also in different tribes. The empodium is hair-like and the pulvilli are developed only in *Culex*, being rudimentary in others. The coxa and the trochanter are very small. The femur is the first visible segment from the dorsum. Both femora and tibiæ are slender and of equal length. The first tarsal segment is as long as the tibia.

Abdomen : The abdomen consists of 9 or 10 segments, the last two being modified for sexual purposes. Within a short time after emergence the tip of the

abdomen in the male mosquito undergoes a torsion of 180 degrees, the true dorsal surfaces of these segments being really ventral surfaces and *vice versa*. The reversed position is probably adapted for the purpose of copulation (Christophers, 1922).

The male genitalia consist of the 9th segment together with its appendages. The whole of this segment is made up by the tergite as the sternite is very narrow. Its appendages are (a) coxites or side pieces to which is articulated ; (b) the clasper ; (c) on the inner side of each coxite is a parabasal lobe which carries parabasal spines ; (d) claspettes or basal lobes lie at the inner basal portion of the coxites ; (e) harpago, a lobe-like process lying on the basal lobes ; (f) phallosome which lies ventrally between the bases of the coxites and carries leaflets.

The anal lobe or the 10th segment lies within 9th tergite ventral to the phallosome.

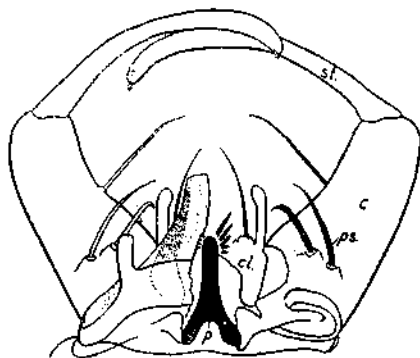


FIG. 18.

Diagram of the male terminalia of an Anopheline imago (dorsal view). (After Christophers).

c, coxite ; cl, claspette ; p, phallosome ; ps, parabasal spine ; st, style.

While the male genitalia are of importance in the identification of the species of *Anopheles*, the female terminalia are not so. The torsion undergone by the male does not take place in the female. The most conspicuous parts are the cerci. The anus opens at the tip of the 10th segment, above and between the cerci. Lying in the 8th segment is a round highly chitinised organ, the spermatheca; it is a single organ in *Anopheles* but consists of three round bodies in *Culex*.

ALIMENTARY CANAL. The stomodæum and proctodæum develop from invaginations of the ectoderm and lining of these regions is, therefore, ectodermal or chitinous in character. The mesenteron is the midgut.

The mouth lies in the region where the mouth parts coalesce and is situated slightly behind the front edge of the clypeus.

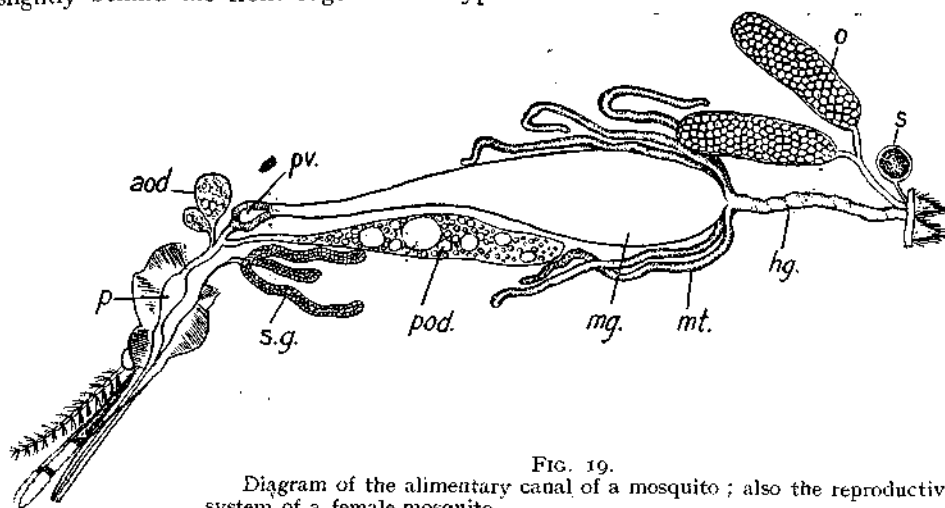


FIG. 19.
Diagram of the alimentary canal of a mosquito; also the reproductive system of a female mosquito.

aod, anterior oesophageal diverticula; hg, hindgut; mg, midgut; o, ovary; p, pharynx; pod, posterior oesophageal diverticula; pv, proventriculus; s, spermatheca; sg, salivary glands; mt, malpighian tubules.

The pharynx is a large flask-shaped organ, the posterior part being widely dilated. The anterior portion is divisible into two regions, the prepharynx and the midpharynx. The posterior portion may be called the postpharynx and constitutes the pumping organ. The dorsal and the ventral walls of the pharynx are continuous with the epi-pharynx and the hypo-pharynx respectively.

The dorsal wall of the prepharynx carries

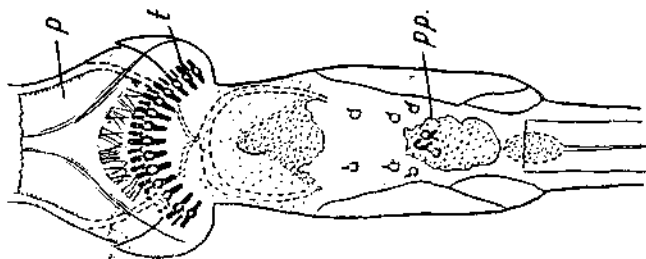


FIG. 20.
Dorsal view of buccal cavity and commencement of pharynx of an anopheline mosquito (after Sinton and Covell).
p, pharynx; pp, palatal papillae; t, bucco-pharyngeal armature.

several palatal papillæ. Below the ventral wall of the prepharynx is a conical organ, the salivary pump. The common salivary duct enters the pump at the broad, posterior end and leaves it at the anterior pointed end to enter the channel in the hypopharynx.

The midpharynx is also provided dorsally with some papillæ. The posterior dorsal wall contains a pigmented area.

On the ventral wall of the postpharynx are some chitinised modified setæ in double rows. These are the pharyngeal armature of Sinton and Covell (1927). These have been used to classify *Anopheles* into five groups (Barraud and Covell, 1928).

The chitinous armature are probably homologous with T-shaped ridges found in the pharynx of saprophagous Diptera larvæ.

The pharynx is provided with strong muscles which assist in suction. At the posterior end it becomes continuous with the œsophagus which is much wider. The œsophagus is provided with three diverticulæ, two dorsal and one ventral.

Œsophageal diverticulæ: The first is small, round and contains a bubble of gas. It is often found collapsed. It is also short that it looks sessile. The second originates from the œsophagus just behind the point of attachment of the first sac. Its shape is at times globular and at times pyriform. The third is elongate with a long duct attached to the œsophagus just anterior to the proventriculus. The sac lies partly within the thorax but mainly within the abdominal cavity, where its lower level may reach as far as the 6th segment; it is often considerably distended with gas. It is only in the ventral sac that peristalsis is quite obvious.

The two dorsal ones are small; the ventral sac is very long and extends into the abdomen sometimes even beyond the 5th segment. They contain minute bubbles of gas and can thus be easily distinguished from the salivary glands. The wall of the diverticula consists of a fine membrane of a chitinous nature together with strands of muscle tissue. It contains no cellular structure.

Function. No satisfactory explanation has hitherto been put forward regarding the exact function of the diverticulæ. The presence of air-bubbles in these sacs has given rise to the supposition that the diverticula provides these insects with the buoyancy required to enable them to undertake long flights in the air. Such air-sacs are, however, wanting in other nematocera.

Nuttall and Shipley (1903) were inclined to believe that these sacs are food reservoirs although it was known that different fluids follow different courses, such as sugar solution and fruit juice finding their way first into the diverticula and then into the stomach, whereas in the case of the blood this order is reversed. Disturbance during the process of feeding, however, does not alter the destination of blood as was thought by Patton and Cragg (1913). The stomach becomes first distended with blood and then the extra amount accumulates in the diverticulæ. (Roy, 1927).

It can, however, be stated that the insect appears to exert a voluntary control in the choice of its food (Mac Gregor and Lee, 1929), though in the matter of directing the food to the different parts of the alimentary canal, it cannot exercise the same control (Roy and Ghosh, 1940).

Salivary glands : The salivary glands, one on each side, consist usually of three lobes or acini, the lateral ones being long and tubular and the middle one considerably shorter. Considerable difference in the structure of the two glands is manifest. The cells of the long acini, according to Christophers, are of granular type and those of the short acini are of colloid type.

The single ducts on each side travel forwards into the neck and head, where they unite to form a common salivary duct. The common duct enters the salivary pump situated below the anterior part of the prepharynx and then opens into the hypopharynx below the region of the "mouth". One of the acini is not infrequently bifid distally and additional or accessory acini are sometimes found (Roy and Mayne, 1931).

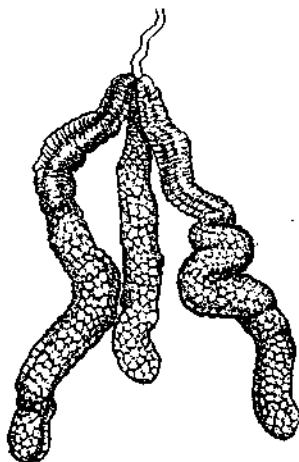


FIG. 21.
Salivary glands of one side separated from the head.

The salivary secretion is in part withdrawn into the stomach with the blood when the mosquito feeds. This is borne out by finding sporozoites intermingled with the blood in the stomach and ventral oesophageal diverticulum of infected mosquitoes shortly after feeding.

Cornwall and Patton (1914) found that the salivary glands contain a powerful agglutinin and that the secretion agglutinates the red cells of the blood. This was confirmed by Yorke and Macfie (1924). The injection of salivary secretion is accompanied by a wheal and the redness is due to subsequent scratching. (Roy, 1927).

Proventriculus : The proventriculus lies at the anterior part of the midgut. It is a muscular organ formed by the invagination of the oesophagus into the stomach. In the mosquito it acts as a muscular valve which prevents the regurgitation of food from the stomach. The midgut or the stomach is elongated, its anterior part being narrow and the posterior part dilated. The mid-intestine, is followed by the hind-intestine and at the junction lies the Malpighian tubules which are five in number. They are excretory in function. The hind-intestine is smaller and much narrower than the mid-intestine. The rectum is provided with six rectal papillae. The anus opens on the ventral surface of the last segment.

REPRODUCTORY SYSTEM. In the female it consists of a pair of large ovaries which when gravid, contain eggs. The ovary connects with a pair of oviducts which are lined with chitin ; they unite to form a common oviduct, its lower part being called the vagina. The number of spermatheca varies in anopheline and culicine mosquitoes. In anophelines only one spermatheca is found, while in

culicines they are usually three in number. The accessory gland is very small and is unpaired.

In the male the internal reproductive organs consist of (a) a pair of testes, (b) a pair of vasa deferentia, (c) a seminal vesicle, (d) ejaculatory duct, and (e) a pair of accessory glands opening into the distal part of the ejaculatory duct.

RESPIRATION. The respiratory exchange is effected by means of a net work of air tubes or tracheæ which ramify through the body. The open externally on the skin. A pair of such openings or spiracles lie one on each side of the thorax.

(For description of larva see page 33).

LIFE HISTORY

The following constitutes the general life history of a mosquito considered on a broad basis.

EGG.

A mosquito passes through four distinct stages, *i.e.*, egg, larva, pupa and adult, the first three being aquatic. Eggs are small dark bodies and are just visible to the naked eye. They are laid on water, leaves and stems of aquatic plants, mud and even on wet-ground where tidal water is likely to reach.

The number of eggs laid by different species varies greatly. The full complement of eggs is generally discharged in one sitting though exceptions occur.

The shape of the egg varies greatly not only in different tribes but also in different species. *Anopheles* and *Aedes* mosquitoes lay their eggs singly, while *Culex* eggs are laid in clusters. The egg is white in colour and is soft but after it has been discharged it turns black and becomes quite hard. Eggs of some species can withstand a considerable amount of desiccation but a large majority of the eggs die when kept away from water for some time.

The egg stage in the tropics under the most favourable conditions does not last more than 2 days.

LARVA.

The egg shell is broken by a head breaker found on the head of the first stage larva. The larva is an active wriggler. It has eyes and it feeds on solids like algæ, organic particles, and microscopic living organisms, and chews with its mandibles. It shows little or no discrimination in the choice of food. By means of the mouth brushes a current of water is set up and particles of food are drawn towards the mouth. The mouth brush also acts as a sieve or filter. From time to time the brushes are cleaned with the comb or pecten situated on the sides of the 8th abdominal segment.

What induces the adult to select a special type of water for breeding is difficult to say. It is no doubt true that some species are more attracted by the physico-chemical properties of the water, others in addition, probably to a special type of food existing in the water.

In mosquito larvæ a proteolytic enzyme is present in the gut which acts in an alkaline medium. Amylase and invertase are also found (Hinman, 1932).

As it is possible to rear larvæ of *Aedes ægypti* in Berkefeld filtered water, it is apparent that mosquito larvæ are able to utilise materials in solution and in suspension (Hinman, 1932). Howland (1930) found that vital stains like neutral red and methylene blue are absorbed with great rapidity by the gut cells and from this it is surmised that the contents of algæ in solution are taken up in the same way.

Anopheline larvæ float just below the surface of water while those of culicine hang from the surface. When alarmed, the larva generally dives to the bottom and remains motionless feigning death. Mosquito larvæ breathe oxygen from the air and while *Anopheles* larvæ cannot remain submerged in water for a long time, *Culex* and *Aedes* can do so.

The breathing apparatus consists of parallel air tubes in the body of the larva which open by a common orifice at the tail end. In the larvæ of *Culex* and *Aedes* the orifice is situated at the end of the prolongation of the air tube, known as the siphon, which is placed at an angle to the line of the body. In *Anopheles* they open on the 8th segment.

Larvæ of both *Anopheles* and *Culex* possess perispiracular glands; these secrete an oily substance which is responsible for the hydrofuge properties of the spiracular region of the mosquito larvæ. The differential wetting of the spiracular region and the action of oil on mosquito larvæ depend on the presence of this oily secretion (Keilin etc., 1935).

The testes in certain *Anopheles* larvæ are often well seen (Adie, 1912).

The larval life of all mosquitoes is interrupted by four stages; at the end of each stage a skin is cast off. It therefore grows by stages.

Anopheline larvæ are not cannibalistic by nature but become so when they are crowded in a limited space. Some culicines, e.g., *Lutzia*, *Armigeres*, and *Megarhinus* larvæ are predators: they also prey on each other.

In the prepupal stage the larva does not feed.

The duration of the larval life varies according to the food supply and temperature. In warm climates this stage may be completed within five days.

PUPA

The pupa is also an active swimmer. It is comma-shaped and sees with its eyes but cannot feed, as the mouth parts are entirely suppressed. Shortly before the adult emerges, it straightens out, and a split appears along the mid-dorsal line of the thorax. The mosquito gradually emerges through this opening, the head first and the legs last. It sits on the empty pupal case for some time and as soon as the body becomes sufficiently hard, it flies away.

The duration of the pupal stage in the different species is somewhat variable. In Calcutta the time taken varies from 26 hours in summer to 48 hours in winter and depends on the seasonal conditions and temperature factor. The imagines emerge mostly between the hours of 6 and 9 in the evening. Emergence may, however, take place in the day light and towards the early morning (Sen, 1935).

All the progeny of a given female will not develop at the same rate neither will all the eggs of a progeny develop into the adult stage. The mortality is the heaviest during the larval stage and as it grows the mortality lessens. Under the most favourable conditions a mosquito may complete the cycle from egg to the adult stage within 9 days and sometimes even less.

ADULT

The posture assumed by a resting imaginal mosquito is to a great extent characteristic of the tribe. Anophelines have a tendency to sit with the head, thorax and abdomen in a straight line, whereas culicines have a hunch-backed appearance. *A. culicifacies*, however, assumes a *Culex* posture while resting.

The proportion of sexes has been stated by some to be equal, whereas others have noted a preponderance of either males or females. Buxton and Hopkins (1927) observed that the sexes are equal in *Aedes*.

Very little is really known about the day-time resting places of mosquitoes. Although they are known to take shelter in the day time in dark and often damp places, it is by no means easy to find them in large numbers. In addition to human habitations and cowsheds they hide in cracks, crevices, tree holes, roof thatch, shrubs, and jungles, not necessarily near breeding and feeding places where they are believed to rest in the day-time. It is always difficult to correlate adult catches in the house with the density of larval breeding, thus suggesting that they generally hide in some unknown places in the day-time. Their infiltration in the dwelling houses at night is influenced by their natural desire for a blood meal and for this reason the males are found in insignificant proportion in such situation. Mosquitoes as a rule seek dark coloured objects for sitting and hiding.

The activity of imagines is influenced by atmospheric conditions. It is thought that the activity is as a rule greatest on dark nights.

Mating is generally assumed to take place on the wing in the open at a considerable altitude above the earth in the evening or at dusk. The males which remain near the breeding place collect in swarms and execute what has been described as a nuptial dance. In some species, however, mating can take place in a limited space even in test tubes. Fertilisation generally takes place from 12 to 24 hours after emergence of the adult.

A mosquito does not feed within 12 to 24 hours after emergence. Though both males and females can live on vegetable sap and carbohydrate food, a blood meal on the part of the female of most mosquitoes is essential for the maturation of the ovum. For this a satisfactory amount of blood is necessary not only for the maturation of the first batch of eggs but also for subsequent batches. As soon as oviposition has been completed, the mosquito is ready to take another blood feed. The intervening period between the ingestion of blood and oviposition is taken for the digestion of the blood meal. In the most favourable season it takes about four days for the maturation of the eggs inside the ovary and in one species of *Anopheles*, two days.

While *A. albimanus* can ingest as much as 0.8 mg. of blood, a full-sized *Aedes* generally takes between 0.3 and 0.35 mg.

The numerical ratio of egg production of a mosquito does not depend on the nature of blood feed whether of man or of animals, but probably on the amount of blood ingested by it. (Roy, 1931, 1936). Not all mosquitoes can be induced to take blood feed in captivity.

When a mosquito once starts feeding it generally feeds to repletion. This is borne out from results of precipitation tests. Only a small-number of individuals are seen to take both bovine and human blood in one night.

Given a substantial blood meal virgin mosquitoes are capable of laying eggs and as these are not fertilised, they will not hatch.

The biting propensity of a mosquito has a definite relationship to the intensity of light, temperature, humidity etc. All mosquitoes show some preferential habits for the blood of man or of animals. The blood found in a gorged female in a human habitation is not necessarily human blood, since the house may be merely a temporary resting place, the meal having been obtained from an animal in the vicinity.

Mosquitoes are primarily attracted to a combination of scent and warmth given off by the human body.

Mosquitoes are known to hibernate, *i.e.*, they pass over from an unfavourable to a favourable season. Hibernation takes place in the winter generally in the adult stage. At this time they do not feed and remain in a semidormant state. It is doubtful if mosquitoes can hibernate in the larval and egg stages; these are really instances of delayed growth rather than of actual hibernation.

During hibernation there is an accumulation of fat and an increase of water in the mosquito. As the fat disappears the space that is occupied is partly filled by an increased amount of air in the diverticula, so that the insect's loss of weight is greater than the reduction in size (Buxton, 1935).

They are liable to be dispersed by railway trains, steamers, aeroplanes, and country boats. Wind may also carry them to distant places though they have a particular dread of boisterous weather.

Comparatively few observations have been made on the longevity of mosquitoes in nature. The life of a mosquito is primarily influenced by temperature and humidity. They generally die when the temperature is high and the humidity low. A low temperature is also fatal to them. The male is as a rule shorter lived than the female.

All insects have a tendency to be attracted to very strong light. On this basis various light traps have been devised to attract mosquitoes which are thus rapidly killed.

The range of flight of a mosquito varies with the species. The males generally do not fly far but the females are able to seek their host by flying a considerable distance from their breeding places.

Natural enemies of adult mosquitoes and their larvæ are numerous. The most important predacious enemies of the adult are lizards, spiders and bats, while those of larvæ are fish. Other natural enemies of larvæ are aquatic beetles and their larvæ, larvæ of dragon-flies and May flies, larvæ of *Chaoborus*, *Mochlonyx*, *Megarhinus*, *Lutzia* etc., small crustaceans, Dytiscid beetles,

Notonecta, tadpoles of the common frog, Diptera larvæ of the family Anothomyidæ and certain molluscs.

Parasites of mosquito larvæ and of adult mosquitoes are also many, e.g., spirochæte, nematode, trematode, ciliate, microsporidia, bacteria, spörozoa, etc., insects and mites.

MOSQUITOES AND DISEASES.

Mosquitoes carry a number of diseases and in this respect they act as true intermediate hosts in which the development of the parasites takes place in their bodies. Human malaria is carried entirely by *Anopheles*, whereas in the spread of filariasis culicines bear the largest responsibility. Some *Anopheles* and *Aedes* have also been accused in this respect. Dengue and yellow fever have long been proved to be carried by *Aedes*. The spread of malaria among birds is effected by culicines. The filariasis in dogs is carried by a mosquito either culicine or anopheline. In this disease the causative agent is a nematode, *Dirofilaria immitis*, the adults of which are lodged in the heart and the microfilariae are only present in the peripheral circulation. The microfilariae enter the stomach of the mosquito during the act of feeding, and migrate to the Malpighian tubules where they rest till the infective stage is reached. The infective filariae are thrown off into the body cavity of the mosquito from which they make their way to the labium. To Grassi and Noè (1900) belongs the credit of having discovered *A. maculipennis* as the intermediate host and since then other mosquitoes have been reported as acting likewise in different countries. *D. repens* which lives in the subcutaneous connective tissue of dogs, and the embryo of which lives in the blood, is carried by *Aedes ægypti* and also by other mosquitoes.

The main points of distinction between the two tribes Anophelini and Culicini are given in a tabular form below.

ADULT

Attitude

Tribe Anophelini	Tribe Culicini
In the resting state the head, thorax and abdomen lie in a straight line. (<i>A. culicifacies</i> is an exception).	The thorax makes an angle with the abdomen, i.e., it has a hunch-backed appearance.

Male

In the male the palpi are clubbed at the end.	The palpi are of uniform thickness throughout the whole length. The terminal part is generally a little bent.
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Female

The palpi in females are as long as the proboscis and are straight.	The palpi are conspicuously shorter than the proboscis.
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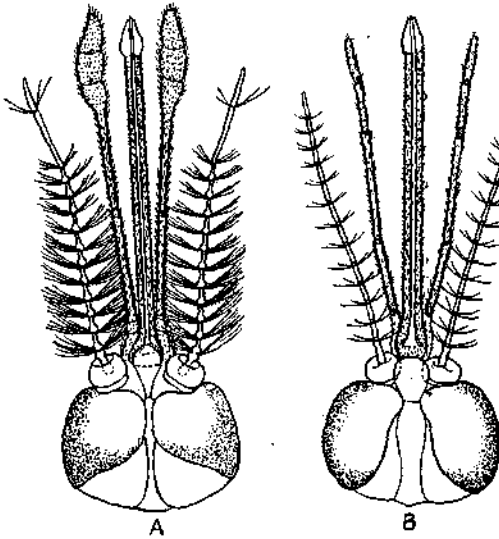


FIG. 22.
Head of male (A) and female (B) *Anopheles*.

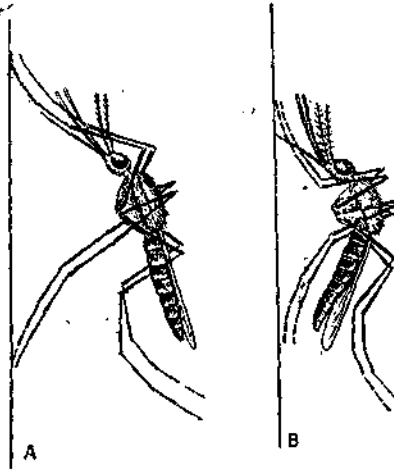


FIG. 24.
The pose of (A) *Anopheles* and (B) *Culex* mosquitoes while resting on wall.

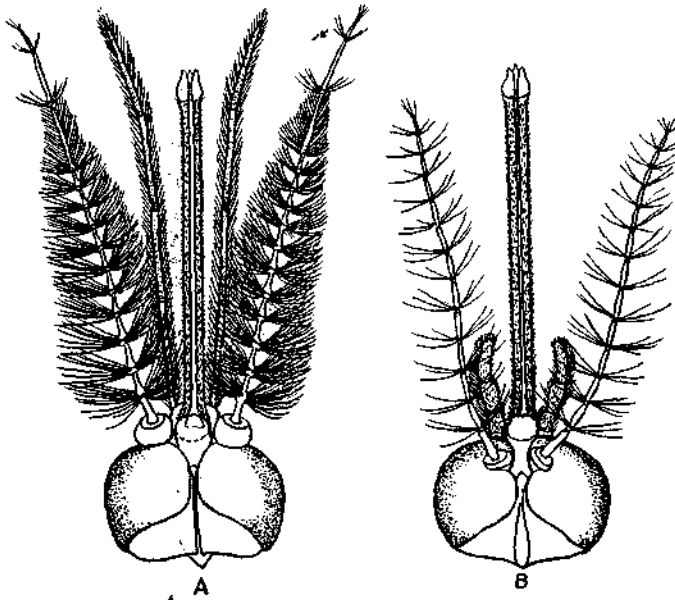


FIG. 23.
Head of male (A) and female (B) *Culex*.

PUPA

The siphon tube is funnel shaped, i.e., short and broad.

The accessory paddle hair lies above the origin of the paddle hair.

The siphon tube is long and narrow.

It is placed beside the paddle hair or may be absent.



FIG. 25.
The pose of resting Anopheline larva.

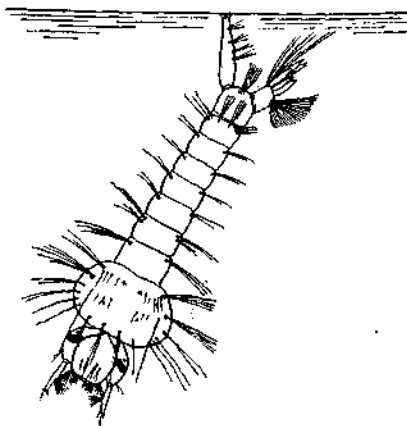


FIG. 26.
Resting attitude of Culicine larva.

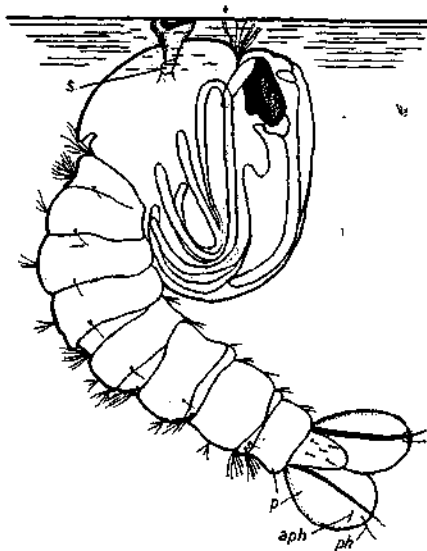


FIG. 27.
Pupa of *Anopheles* mosquito.
aph, accessory paddle hair; p, paddle;
ph, paddle hair; s, siphon tube.

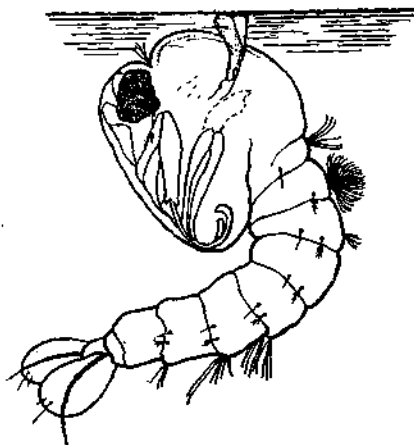


FIG. 28.
Pupa of *Culex* mosquito.

LARVA

It floats parallel to the surface of water.

It has a characteristic swift movement.

Siphon tube absent.

The respiratory openings lie on the 8th abdominal segment.

Palmate hairs, arranged in pairs, are present on many abdominal segments and sometimes on the meta-thorax.

It hangs from the surface of water.

It has a much slower and snake-like movement.

Siphon tube present.

They lie at the end of the siphon tube.

Palmate hairs are absent.

EGG

It is boat-shaped and is provided with lateral floats.

Laid singly.

Elongated.

Either laid singly (*Aedes*, *Armigeres* etc.), in rafts (*Culex*), or in a cluster (*Mansonioides*).

ANOPHELINE LARVA.

The body is divided into head, thorax and abdomen.

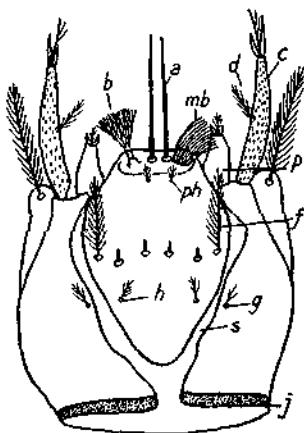


FIG. 29.

Head of *Anopheles* larva (dorsal view).

a, anterior-internal clypeal hair; b, anterior-external clypeal hair; c, antenna; d, antennal hair; f, frontal hair; p, maxillary palp; ph, posterior clypeal hair; s, occipital suture; g, external sutural hair; h, internal sutural hair; j, collar; mb, mouth brush.

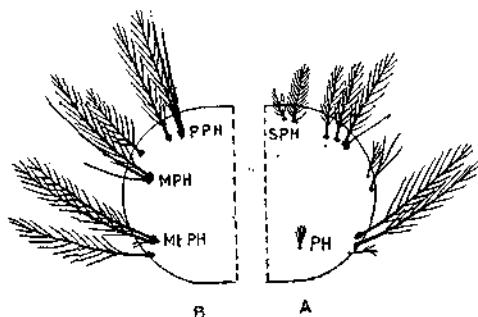


FIG. 31.

A. Dorsal view of the right half of the thorax of *Anopheles* larva.

B. Ventral view of the right half of the thorax of *Anopheles* larva.

M.P.H.—Mesothoracic pleural hairs.

Mt. PH—Metathoracic pleural hairs.

PH—Metathoracic palmate hair.

PPH—Prothoracic pleural hairs.

SPH—Submedian prothoracic hairs.

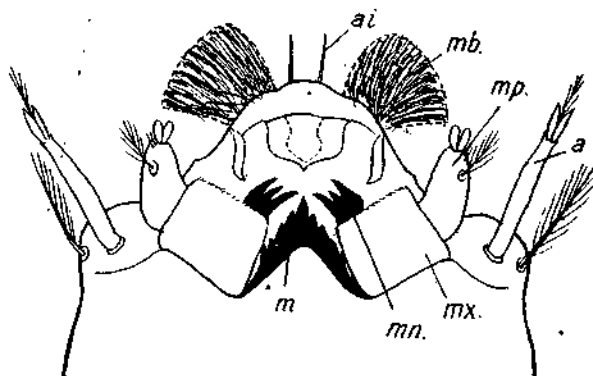


FIG. 30.

Ventral view of the anterior part of head of an anopheline larva.

a, antenna; ai, anterior-internal clypeal hair; m, mentum; mb, mouth brush; mn, mandible; mp, maxillary palp; mx, maxilla.

Head: The median frontal plate on the head is known as the fronto-clypeus which is roughly triangular in shape. The eyes are large and reniform. The following organs constitute the appendages of the head.

1. A pair of antennae which gradually taper from the base to the apex.
2. Mouth brushes, one on either side of the middle line.

3. A pair of toothed mandibles.
4. A pair of maxillæ which are rectangular in shape.
5. To each maxilla is attached a palp which lies internal to the antenna. The palp is much shorter and broader than the antenna.

6. A median organ, the mentum, which bears lateral teeth.

The mouth lies between the mandibles laterally and the mentum posteriorly.

Hairs of the head : Only those which are of taxonomic importance are given.

(a) Clypeal hairs: Anterior-internal, anterior-external and posterior hairs.

(b) Frontal hairs: There are three pairs of frontal hairs arranged in a row and they are generally branched.

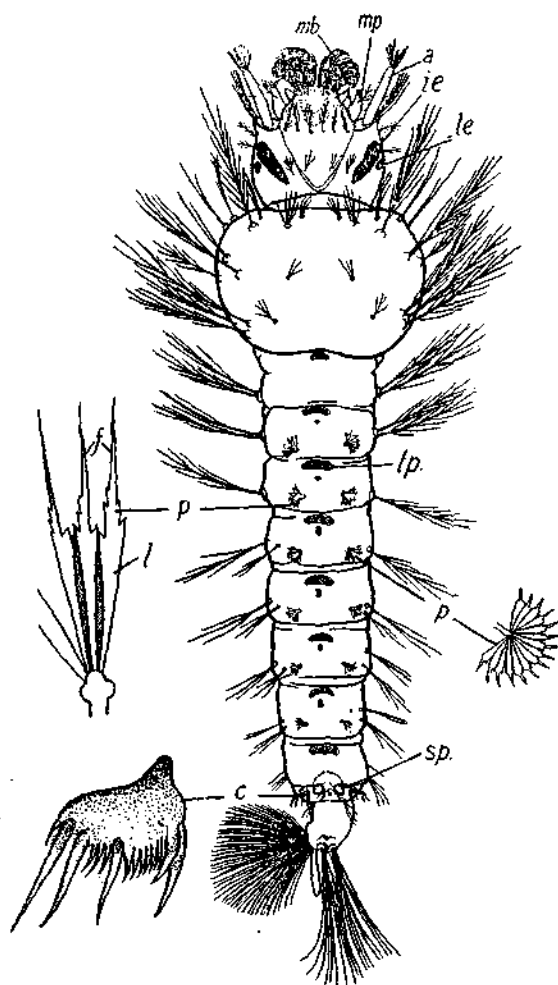


FIG. 32.

Anopheline larva (fourth stage).

a, antenna; c, comb; f, filament; ie, imaginal eye; l, leaflet; le, larval eye; mb, mouth brush; mp, maxillary palp; p, palmate hair; sp, spiracle; tp, anterior tergal plate.

(c) Internal sutural hairs: They arise on each side of the fronto-clypeus.

(d) Outer sutural plate on either side on each epicranial plate on either side of the epicranial suture.

Thorax : Although the thorax is fused, its segmented character is indicated by the arrangement of pleural hairs which lie on its ventral surface and are known as the prothoracic, mesothoracic and metathoracic pleural hairs. On the dorsum of the prothorax is a group of three hairs called submedian prothoracic hairs. The palmate hair may or may not be developed. It is present on the dorsal aspect of the metathorax.

In the living larva a notched retractile organ will be seen occasionally protruding from the antero-lateral region of the thorax.

Abdomen : The abdomen is composed of 10 segments but only 9 are visible. On the dorsal surface of each segment

near the anterior border is a dark oval plate called the anterior tergal plate. Posterior to the anterior tergal plate is a small rounded plate, the posterior tergal plate. At the postero-lateral region on the dorsum of certain abdominal segments lie the abdominal palmate hairs, one on each side of the middle line. They are so called on account of their resemblance to palm leaves. Each palmate hair is composed of a number of leaflets, each leaflet being composed of a blade and a filament. On the 8th and 9th abdominal segments lie the spiracular apparatus, the spiracular openings being found on the eighth segment, and on each side of this segment is a chitinous plate possessing spines of varying length and called the pecten. In addition to a large number of hairs, the last or anal segment carries four membranous organs called anal gills.

CHARACTERS OF THE DIFFERENT STAGES OF ANOPHELINE LARVÆ.

First stage :

- (a) Extremely small, even minute.
- (b) When compared with 4th stage larva, the head is much longer.
- (c) The collar in the neck is very broad.
- (d) The presence of the egg-breaker which lies on the posterior part of the head in a small depression in the middle line is characteristic of the first stage larva.
- (e) All hairs on the head are simple.

Second stage :

- (a) Egg-breaker is absent.
- (b) It is larger in size.
- (c) The shape of the head gradually approaches that of the fourth stage.
- (d) Hairs on the head show very minute branching.

Third stage :

- (a) Resembles fourth-stage larva but smaller in size and is considerably thinner.
- (b) Collar is broader than that of the mature larva.
- (c) The hairs show more branching than in the second stage but decidedly fewer branches than in the fourth stage.

Fourth stage :

- (a) Attains a still larger size and looks quite plump.
- (b) At this stage the imaginal eye makes its appearance.
- (c) Collar is distinctly narrower than in the third stage.
- (d) The branching of the hairs on the head are well developed.

Tribe Anophelini.

This tribe contains only one genus *Anopheles* though Edwards (1932) regarded the tribe as containing three genera, *Bironella*, *Chagasia* and *Anopheles*. The first is confined to New Guinea and the second to the New World. The genus *Anopheles* is found in both the Eastern and Western hemispheres of the globe.

This genus has been divided by Christophers into four subgenera on the basis of genital characters and pharyngeal armature. Among them only two subgenera occur in the old World, *Anopheles* and *Myzomyia*. The former has less than four main costal spots on the wing and the pharyngeal armature is absent. In *Myzomyia* there are four or more costal spots and the pharyngeal armature is present.

GENUS ANOPHELES.

LIFE HISTORY:—Certain special features in connection with the life history and biology of Anopheline mosquitoes are given below:

EGG

Anopheline eggs are always laid separately; they float on the surface of water. They are usually boat-shaped, the posterior extremity being narrower than the anterior. The upper surface or the deck is flat and corresponds to the ventral aspect of the contained larva. Surrounding the upper surface is the frill and at the sides of the egg are the floats which contain air and which help the egg to float on the surface of water. The frill is usually striated and shows a number of corrugations. The majority of the oriental species have the egg conforming to one of two main types, the whale-back and the lifeboat-shaped egg. Eggs should be studied under the microscope on wet cotton-wool. They may be preserved for an indefinite length of time in 4 per cent. formalin solution. They may also be mounted in glycerine and the margin of the cover-glass ringed.

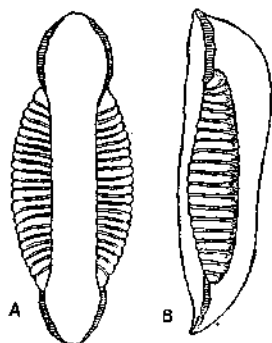


FIG. 33.
Eggs of *Anopheles philippinensis* (after Roy and Siddons).
A. Dorsal view; B. Lateral view.

Blood and egg formation.

So far as is known all anophelines require a blood meal for the maturation of their eggs. According to Darling (1910) *A. pseudopunctipennis* or *A. albimanus* takes approximately 0.8 mg. of blood.

What portion of the different constituents of blood is directly concerned in the formation of eggs is not known. It is, however, known that the protein element in the blood after absorption exercises two-fold functions on the ovaries. While one part acts directly on the follicles stimulating them to activity, the other part provides actual material for the formation of the egg. The earliest change in the egg is characterised by the appearance of light peppery granules, and later by the formation of yolk.

A curious feature in the development of eggs in *A. subpictus* is the fact that in addition to blood feed, reception of spermatozoa in the receptaculum seminis is also necessary. This is not seen in any other *Anopheles* so far studied. (Roy, 1940).

Development of eggs: While the intraovarian life of eggs in Calcutta is 4 days (Roy, 1940), Christophers (1911) found it to be 6 in the Punjab. The

chronological succession of stages noticed in Calcutta in its development is given below.

Stage I up to 24 hours: Complete filling up of the ovum with yolk granules.

Stage II up to 48 hours: Elongation of the ovum.

Stage III up to 72 hours: Appearance of floats.

Stage IV up to 96 hours: Hardening of the cuticle and maturation of the ovum.

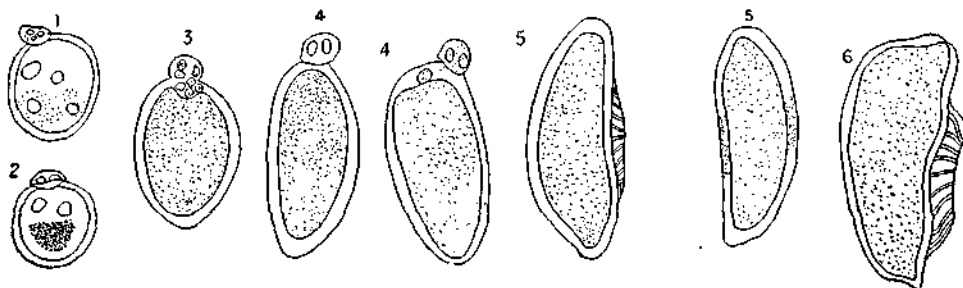


FIG. 34.

Development of eggs of *Anopheles subpictus* (after Roy).

1. Showing light peppery granules distributed irregularly indicating stimulation of the follicle. 2. Showing deposition of yolk granules. 3. Stage 1 up to 24 hours. 4. Stage 2 from 24 to 48 hours. 5. Stage 3 from 48 to 72 hours. 6. Stage 4 from 72 to 96 hours.

Anopheline eggs, like eggs of other mosquitoes, undergo some increase in size after they are laid, due to absorption of water.

Number of eggs: In Malaya the average number of eggs in different species of *Anopheles* has been recorded by Lamborn (1922). The number was noticed to vary from 29 found in *A. tessellatus* to 89 in *A. vagus*. Similar information in respect of Indian *Anopheles* is meagre. In *A. stephensi* this was found to be on an average 80. Up to three batches of eggs have been recorded and the maximum longevity has been noticed to be 5 weeks, the female producing 5 batches of eggs during this period (Roy, 1931). *A. minimus* produces 118 and *A. maculatus* 147 in one laying. (Viswanathan, 1941).

LARVA

Almost every species of *Anopheles* has some particular type of breeding place which it prefers above all others and which is essential if it is to flourish to any extent. From this the selective breeding habits of most of the common species has been determined. Some species are found in slowly running streams, some are swamp breeders and among them are those that prefer open swamps, while others prefer shade. A number of species are very fond of temporary pools, open cisterns, and collections of rain-water. Some like *A. subpictus* breed in extraordinary profusion in the little muddy pools. *A. sudaicus* is always found in brackish water.

Mosquito breeding in any type of water was once regarded as a physical phenomenon. The explanation of different species selecting particular types of water is now sought in the physical and chemical constituents of water. The algae,

planktons and micro-organisms all play their respective rôles in the choice of the water by the female for egg-laying.

It has been suggested by Williamson (1927) that chemical characteristics may be among the ultimate dominants of mosquito breeding. Specific distribution of Anophelines, correlated in relation to dissolved oxygen and mineral solution, has been stressed by Senior White (1927).

The importance of food in the ecology can not be underestimated. Certain types of algae and plants have been supposed to attract a particular type of mosquito, while negative associations have also been recorded (Senior White, 1925). Buxton and Hopkins (1925) showed that water vapour by itself was not highly attractive to the female *Aedes aegypti*, but that water to which various organic substances had been added induced many more layings and eggs.

Micro-organisms too are known to induce mosquitoes to oviposit ; these serve as larval food (Lamborn, 1922).

The duration and intensity of radiation falling upon the water surface are also important, as both the direct and indirect rays of the sun have considerable effect both on the production of food used by the larvae, and on the temperature of the water.

Method of feeding : The anopheline larva is exclusively a surface feeder. At the time of feeding the head rotates through 180 degrees so as to bring the ventral surface uppermost. With the help of the feeding brushes a current is set in motion whereby the food materials are drawn into the mouth. Large particles of solids are rejected. This, according to Christophers and Puri (1929), is the process of free-feeding. The other method of feeding is the film-feeding. Here the larva subsists on bacteria and animal organisms floating on the surface of still water.

Drifting of larvae : Often an incredibly large number of mosquito larvae are carried by the current of streams. Such drifting larvae may have a serious effect in disturbing the results of anti-mosquito measure (Sinton and Majid, 1935).

PUPA.

In the great majority of instances both pupation and emergence of the adult take place during the night. The specific identification of *Anopheles* pupa can often be made from the hairs on the abdomen.

ADULT

The period from oviposition to the emergence of the first offspring varies in different mosquitoes, the earliest being about 9 days.

Mating : Very little is known of the mating habits of Indian Anophelines. Rao and Russell (1938) have recorded mating in *A. annularis* by swarming. Rao, Roy, and Rao (1942) have also noticed this in *A. sondaicus* and *A. annularis*. It is believed that *A. maculatus*, and *A. minimus* do not usually mate in confinement (Viswanathan *et al.*, 1941).

Food preference: Anophelines as a rule show a decided preference for either human or animal blood. The hypotheses advanced on the food preferences of Anophelines are the following: (1) the choice of the food is determined by the maxillary index of the *Anopheles*, (2) the odour of the animal attracts the particular species of *Anopheles*, and (3) feeding is induced by favourable micro-climatic conditions. It is very doubtful that the behaviour of anophelines towards man is determined by their maxillary armature. The consensus of opinion is that the particular attraction towards either man or animal is determined not so much by the paucity of teeth on the maxillae as by the odour. The availability of the proper host is also important.

Resting habits: After feeding, the activity of the mosquito is greatly curtailed, when it rests in some sheltered place for the purpose of digesting the blood and for development of eggs. In practice what is usually noticed is that while a very large number of *Anopheles* may be caught at night, they are scarce in the day time when only a few are seen resting in dwelling houses and cowsheds.

Dispersal of Anopheles: The carriage of *A. gambiae* from Africa to Brazil where it has proved a great menace is an example of mosquitoes being transported in aeroplanes. (Shannon, 1932). In India the presence of *A. sundaius* in Calcutta is another example where they were carried from the coastal to inland areas by railway trains and country boats. (Senior White, 1937). Similar dispersal of *Anopheles* has been noticed in other countries. Newstead and Carter (1927) found *Anopheles* mosquitoes in ships arriving in the port of Liverpool from West Africa.

Flight: The distance a mosquito can fly is of immense practical importance, especially in the case of malaria-carrying species.

This is generally determined by breeding them out in cages, and then staining them with an aqueous solution of an aniline dye. An atomiser is used to direct a fine spray upon the mosquitoes. Majid (1937) has found "gold" powder, commonly used in press work, extremely useful for marking mosquitoes. The powder is dusted after the insects have been lightly anaesthetised. The dusts are detected with the aid of the microscope. After a period of rest the mosquitoes are liberated to test their range of flight.

Anophelines can often cover a long distance by their own activities, either by a single flight or a succession of flights. The intensity of their breeding also influences the extent of their dispersion. Much also depends on the proximity to the breeding places of suitable sources of food and shelter and also on the presence of natural barriers such as a large sheet of water, or high hills.

In Malaya a distance of half-a-mile is considered the maximum effective range of *A. maculatus*. In the Netherlands East Indies a control area of one and a half miles is considered necessary. In the latter country *A. sundaius* is the chief danger and this mosquito is strong on the wing. In the Philippines *A. funestus* is believed to migrate a long distance from the breeding place, possibly more than 1.5 kilometers. Barber and Hayne (1925), Fisher (1923), Kligler (1924) have all pointed out that anophelines may even disperse and fly a distance greater than 1 mile. Kligler and Mer (1930) have published evidence that *A. elutus* covers

distances up to 5 miles during its hibernation flight while the range at other times is a little over a quarter of this distance.

It is, however, thought that for all practical purposes a flight range of half a mile may be fixed for *Anopheles*. Though it is impossible to fix, it may be safely asserted that few individuals ever stray much more than a quarter of a mile from the pool in which their larvæ are found. In spite of popular belief to the contrary, they may be carried far by the wind even 10 miles as has been recorded by Wenyon (1926). Napier (1940) has pointed out that *A. gambiae* can fly a long distance from the African coast to ships anchored at sea and can even transmit malaria to passengers.

Hibernation : In the case of *Anopheles*, hibernation generally occurs in the adult female. It is also thought that some hibernate in the larval stage.

In India where the temperature is quite favourable for mosquito breeding throughout the year, hibernation is extremely uncommon, although its occurrence has been suggested by a number of workers. From laboratory studies alone Chowdhury (1931) thought *A. subpictus* to be a truly hibernating species in the Punjab.

In regard to *A. culicifacies* the probability is that in the cold regions in the Northern Punjab and N.W.F. Province, this species passes the winter in the larval form. It does not hibernate either in Sind or in the Punjab.

Length of life : The length of life of the adult female *Anopheles* is an important factor in the epidemiology of malaria. The longer the *Anopheles* female lives, the greater chance it has to become infected and infective.

The transmission of malaria as also the length of life of the mosquito are dependent upon the co-existence of certain conditions of temperature and humidity.

Atmospheric humidity favours the transmission of malaria in three ways:—

- (1) By prolonging the life of the mosquito till the sporozoites appear in the salivary glands ;
- (2) The development of the parasite in the insect host is dependent on the influence of the prevailing temperature and humidity ;
- (3) The biting propensity of a mosquito has a definite relationship to, temperature and humidity.

While a hibernating mosquito may live for six months or more, it is extremely doubtful if the ordinary *Anopheles* in India, at least in the plains, can live much longer than a month, the majority dying much earlier.

Effect of malaria parasite on the mosquito :

Various opinions have been expressed as to whether a malaria parasite of man has any harmful effect on the *Anopheles* in whose body it grows and multiplies. It is commonly thought that the infection is not injurious to the insect. Buxton (1935), on the other hand, has shown that death occurs earlier among *Culex fatigans* infected with *Proteosoma* than among the control mosquitoes, the reason for which he directly ascribes to the invasion of the wall of the midgut by the ookinete.

Age : Perry (1912) divided *Anopheles* into four grades according to the condition of the wings. In grade I are placed specimens with the wing well

marked and the wing fringe practically complete ; in grade 2 those with the wing fairly well marked, but the fringe somewhat worn ; in grade 3 those with the wing decidedly shabby, and the fringe very much worn ; in grade 4 those with the wing actually threadbare. The largest percentage of infection of the salivary glands is generally found in grades 3 and 4 mosquitoes.

The multiparous ovary can be distinguished from the nulliparous. Mer (1932) has found distinct changes in the common oviduct in *A. elutus* after eggs have once been discharged. The presence of retained ova, a condition not commonly encountered, points to the mosquito being multiparous. Also a multiparous ovary has a different appearance from that seen in the nulliparous condition.

Parasites : The parasites which are known particularly to attack *Anopheles* larvæ and adults are *Microsporidia*, certain *Mermithids* and fungi of the genus *Coelomomyces* of which 2 species have been described by Iyengar (1938), *C. indiana* and *C. anophelisica*, both of which infest *Anopheles* larvæ and adults and cause considerable mortality. Three genera of *Microsporidia*, *Thelohania*, *Nosema* and *Plistophora*, have also been recorded by him as attacking larvæ. The life cycle of Mermis (genus *Thelohania*), parasitic in *Anopheles* larvæ has been traced by him (1929). Kudo (1929) and Sen (1941) have described four species from Indian anopheline larvæ. Sinton (1932) recorded a list of trematode infection found in Indian anopheline mosquitoes.

Several genera of mites belonging to the family Hydrachnidæ are found as ectoparasites of adults. The forms found on mosquitoes are the larval stages of these acarines ; their adult stages are aquatic and free-living.

Only one insect, *Culicoides anopheles*, is known to attack the female *Anopheles*. It has not been recorded from the male or from any culicine. It seems that the *Culicoides* sustains itself on the blood ingested by the mosquito.

RACES OR VARIETIES OF ANOPHELES.

The presence of different races within *A. maculipennis* was first suggested by Roubaud from the fact that one of them had lost touch with man. In 1921 he proposed a method of distinguishing the races according to the number of maxillary teeth. Those that favour cattle were thought to be provided with a strong dental armature as opposed to a weak one in those which prefer to bite man. In 1924 Fulleroni drew attention to the existence of a difference in the markings of certain eggs from others and simultaneously van Thiel in Holland showed that the females of *A. maculipennis* were of two varieties, one short-winged and the other long-winged. In this way the existence of six different races or varieties of *A. maculipennis* was clearly recognised. They showed structural, biological and physiological differences and among them only two, var. *labranchiæ* and var. *elutus* have been found to be dangerous vectors of malaria ; others were associated with malaria transmission only under exceptional circumstances. In the laboratory, on the other hand, no differences in their susceptibility to malarial infection have been found.

The conception of races in the Indian species has arisen from the diverse physiological behaviour on the part of the same mosquito in different localities and often in contiguous localities.

The following may be taken as exhibiting varieties or races within the species:

A. stephensi, *A. culicifacies*, *A. philippinensis*, *A. maculatus*, *A. aconitus*.

A. stephensi: Recent researches of Sweet and Rao (1937) have established without doubt that two distinct races or varieties are comprised in *A. stephensi*. No difference in their vector potentiality has, however, been discovered.

In Calcutta, both varieties of *A. stephensi* exist, and possibly in equal proportion; but when subjected to precipitation tests they showed a great tendency to feed on bovine rather than on human blood. (Roy, Chandra and Siddons, 1939). Nevertheless, the recent outbreaks of malaria in the centre of this city has been definitely traced to this species. (Siddons, 1943).

A. culicifacies: In consequence of the finding of salivary gland infection in *A. culicifacies* in the Punjab and in other parts of India it was assumed that *A. culicifacies* was a homogeneous species and its behaviour in regard to its infective potentiality would be constant wherever it was found. It is now known that in such notoriously malarial places as Assam, Bengal, Jeypore Hill tracts and Singbhum, it has been found to be totally innocuous.

A. philippinensis: This is another species whose malarial transmitting potentiality is not everywhere constant. Although this species has been reported from Bengal, Assam, Southern India, Burma and Indo-China, infection has been recorded only in Bengal and Burma, the infection rate in Bengal being quite high. It is considered as the chief vector in the plains of Bengal.

A. maculatus: It is claimed that *A. maculatus* in India is different from the Malayan form where its power to transmit malaria is well known. In India, on the other hand, although it is common in all hilly areas, it appears to be a non-vector, except in Shillong town. There are indications, however, that this species is primarily a zoophilic race in India. Its anthropophilism in Malaya may be due to the virtual absence of cattle from that country.

A. aconitus: This mosquito has a wide distribution in the Oriental Region; in India it has been recorded from the eastern and southern areas. Although it has been considered to be a dangerous species in the Dutch East Indies and in Malaya, the Indian variety has so far been regarded more or less innocuous except in one locality in Bengal (Das, 1943).

ANOPHELES SPECIES

Nearly 42 species with 10 varieties representing local forms or subspecies have been recorded from the Indian area. From their male genital characteristics, this genus has been further subdivided into 3 subgenera, *Anopheles*, *Nyssorhynchus*, and *Myzomyia*, the first and third of which are found in India.

ANOPHELES OF INDIA.

A. culicifacies Giles.

A. culicifacies has a very wide distribution in India, Burma, and Ceylon. It is the most important malaria-carrying species in India except in Bengal, Assam and the Jeypore Hill Tracts (Senior White, 1937). In Bengal it is prevalent during the pre-monsoon and monsoon period (Krishnan, 1937) and during the winter in Assam. In the Punjab it is most numerous in October and in certain regions during the early winter months. It is undoubtedly the species concerned in epidemic malaria.

The characteristic feature about this species is its *Culex*-like colour and *Culex*-like attitude. *A. culicifacies* has a tendency to hide itself in well-sheltered places, such as in holes, among dung cakes, in chaff etc. In Ceylon it penetrates a long way into the thatch of cow-houses, and even into the bristles of a nail brush (Clemesha, 1934).

Although Timbres (1935) found this species relatively more prevalent in domestic houses than in cowsheds in Bengal, the experience of different workers is that the proportion found in cowsheds is considerably much higher.

They enter dwelling houses to feed in the middle of the night (Afridi and Puri, 1940).

The results of precipitin tests indicate that this species is more zoophilic than anthropophilic (Afridi, Singh and Singh, 1939). Nevertheless Nursing *et al.*, (1934) found that they were caught in larger numbers in human baited tents than in houses and cowsheds in Mysore.

It cannot be colonised and is slow to take blood feed in captivity. The sporozoite index in this species is generally low. It is, however, extremely susceptible to artificial infection in the laboratory.

A. culicifacies is ubiquitous in its selection of breeding places but generally prefers clear water. It breeds in open irrigation channels, river beds, pools, ponds, lakes, springs, in leaks and back water, borrow-pits, in fruit and vegetable gardens, and even in wells. The larvæ are also found in seepages and other collections of still water in which grassy vegetation is abundant. It may occur in rice fields when the plants are very small, also in fallow lands.

It is commonly believed that *A. culicifacies* does not ordinarily disperse to distances much in excess of half a mile. But where food and shelter are scarce, this distance may be extended. Clemesha (1934) found that under certain circumstances it is able to travel even two miles in search of food. In hilly countries it will travel long distances up and down ravines in search of human beings or animals.

Adult *culicifacies* can survive for 8 weeks in the laboratory under winter conditions and the length of the larval stage may be prolonged from 80 to 105 days. Lowering of temperature within the range 95°F to 70°F has a favourable influence on the survival and longevity of this species. Though increase in humidity is beneficial, yet 100 per cent relative humidity is detrimental (Pal, 1943).

According to Afridi, Majid and Shah (1940), *A. culicifacies* can survive up to 56 days in November and December, the life in other months being considerably shorter.

The upper limiting value of temperature for development of Indian strains of *Plasmodium vivax* and *P. falciparum* in *A. culicifacies* lies about 90°F. The optimum range of temperature for development of the parasites and transmission is from 70°F to 86°F, provided humidity is favourable for survival of *A. culicifacies*. At 59.6°F, oöcysts of *P. vivax* and *P. falciparum* have been noticed to develop (Siddons, 1944).

A. minimus Theob.

It is very widely distributed in the Oriental Region. In India it is found in Assam, Dooars and Southern India.

It is a dangerous malaria carrier wherever it is found in large numbers. The sporozoite index in this species is generally high.

In Assam it breeds throughout the year in perennial streams close to human habitations. The larvæ are also found in irrigation channels, seepages, slow-moving drains, tanks, rice-fields, borrow-pits, etc. It does not breed in low-lying areas flooded by water with silt or clay in suspension. Two essential requirements for its breeding are clear, unpolluted water in motion and grassy edges.

The following is a summary of the habits and bionomics of *A. minimus* recorded by Thomson (1941).

The adult has a tendency to rest in the day time in dwelling houses and shows a decided preference for feeding on human blood.

At sundown there is a strong attraction to light and most mosquitoes leave the house at that time. There is a considerable daily turn over of the population in houses. About 90 per cent. of blood feeding takes place after midnight.

During the hot damp monsoon *A. minimus* takes two days to digest its meal, and develop its ovaries, ovipositing on the second night after feeding. In cold weather conditions this period is increased from four to six days. After oviposition *A. minimus* returns for another blood meal on the same night.

Oviposition normally takes place at night and 69 per cent. of eggs are laid in the first three hours of the night. Larvæ are most abundant where the grassy edge is dense enough to produce a certain amount of shade. Though Thomson found shaded areas more attractive for oviposition, in nature the breeding at once ceases in the absence of sunlight.

The flight distance, according to Ramsay, is one mile (1929).

A. minimus is an important malaria-carrying mosquito in Burma, Indo-China, Southern China and Yunnan.

A. fluviatilis James.

It is essentially an Indian species, and outside the Indian area has been recorded only from Siam, Tonkin and Turkestan.

It has a wide distribution in India especially in foot-hill areas and hilly and rocky tracts. It closely resembles *A. minimus* in its habits, its larvæ being found

in running streams. Its importance as a carrier of malaria has been stressed by Senior White (1937, 1938, 1943), and Senior White and Adhikari (1940). It is the principal carrier in the Singhbhum hills, in Hazaribagh and Ranchi plateau. It is also a proved carrier in many places in southern India including Mysore State (Nursing *et al.*, 1934), and Nilgiris District (Russell and Jacob, 1942). It prefers to rest in human habitations in the day time. The adult enters a house in the first part of the night after dark (Viswanathan and Rao, 1943), and also both in the first and second half of the night (Nursing *et al.*, 1934).

A. varuna Iyengar.

The adult of this species was first described by Iyengar (1924) from specimens collected in Bengal. It has now been found to have a wider distribution in Bengal, Peninsular India and Singhbhum Hills.

It breeds in stagnant fresh water, in ponds and ditches and is most prevalent during and soon after the monsoon when it breeds in collections of storm water by the road side. It generally prefers shade, *e.g.*, overhanging branches of trees.

Natural infection has been recorded from lower Bengal (Iyengar, 1928; Roy, 1939). It is an important carrier of malaria in the Jeypore Hills (Senior White, 1937), in the Singhbhum Hills (Senior White and Das, 1938), and in the eastern Satpura range (Senior White and Adhikari, 1940).

It is more attracted to human habitations than to cattle sheds, and overflows to cattle sheds only in the peak months (Senior White, 1937). This species does not leave the house at dawn.

A. sundaicus Rodenwaldt.

A. sundaicus is distinguished from *A. ludlowi* by the phallosome. True *A. ludlowi* occurs in the Philippines and Formosa, the Indian species being *A. sundaicus*. The latter is also found in the Andamans, Malaya Peninsula, Burma and Sumatra. In fact it is widely distributed in the Oriental Region occurring along the sea coast. In India it is found on the coastal regions of Bengal, Orissa and Andamans, and is responsible for malaria outbreaks in these localities (Iyengar, 1931; Senior White and Adhikari, 1939; Covell and Singh, 1942; Panigrahi, 1942; and Christophers, 1912).

It breeds in brackish water generally in association with floating algae such as Enteromorpha, Oscillatoria, Hydrilla, Spirogyra and Chaetomorpha (Sen 1938). It will also breed in water in which no algae are present, or at any rate none visible to the naked eye. This species is distinctly alkaliphile.

A. sundaicus unlike the majority of malaria-carrying species can withstand a considerable degree of organic pollution in its breeding places. Indeed, according to Rodenwaldt and Essed (1925), it actually favours places which are heavily polluted with urine and faeces.

Opinions vary as to the optimum amount of salinity for its breeding. Rodenwaldt and Essed (1925) in the Dutch East-Indies considered that from 1.2 to 1.8 per cent was the most favourable concentration, whilst Iyengar (1931) found it to be 0.15 to 0.26 per cent., and Christophers (1912) 0.4 per cent.

It has a tendency to bite man whenever it has the opportunity to do so and is found both in cattle sheds and in domestic houses.

The outbreak of malaria in relation to this mosquito takes place suddenly and spreads very rapidly. This is invariably associated with rapid increase in its breeding.

The estuarine regions of the sea coast are relatively free from malaria, but the clearing of the virgin mangrove forests in these areas has resulted in a greater incidence of this disease, due to increased breeding of *A. sundanicus*.

A. stephensi Liston.

It is essentially an Indian species and has been recorded from outside the Indian area only from Arabia and Mesopotamia. This species has a wide distribution throughout the whole of India including upper Burma and Yunnan, but has not been recorded from Ceylon.

It is normally an urban mosquito though occasionally also found in the rural areas. It is a highly efficient malaria carrier and is responsible wholly or partly for malaria in cities like Bombay, Madras, Bikaner, Calcutta, Quetta, Sind, Kohat, Mysore, etc.

It prefers small collections of clean water for ovipositing, such as overhead cisterns, wells, small water reservoirs, pools, springs, marsh, and collections of rain water. It often breeds in large numbers in river beds. In fact it is found in a wide variety of breeding places. The larva does not always require aquatic vegetation for its food. It can even adapt itself to saline water.

A. stephensi has been separated into two races on the evidence of the size of eggs, wing lengths and the character of the offspring (Sweet *et al.*, 1938 ; Rao, Sweet and Rao, 1938). The claim of Sweet and Rao (1937) that there is a difference in the malaria carrying potentiality between them has not been substantiated by Russell and Mohan (1939) who found both types equally susceptible to infection in the laboratory. Senior White and Rao (1943) have associated outbreaks of malaria epidemics with *A. stephensi* var. *mysoriensis* in places around Vizagapatam.

In Calcutta the adults are difficult to catch and prefer to rest in cowsheds in the day time (Strickland and Roy, 1933). It is generally believed that this mosquito does not disperse to long distances from its breeding places. Siddons (1943) has been successful in finding infected specimens of this species in dwelling houses in Calcutta where one member after another was attacked with malaria thus suggesting that *A. stephensi* lives in the house after a blood meal.

It is extremely susceptible to artificial infection in the laboratory.

Knowles and Basu (1936) recorded infection in this species with *P. vivax* occurring at 60 to 90°F and humidities between 70 and 100 per cent. The heaviest salivary gland infections were obtained at 80°F, but at 100°F and humidities between 50 and 100 per cent, *A. stephensi* does not survive long enough to become infective. Infection occurred with *P. falciparum* between 70 and 90°F and humidities between 50 and 100 per cent. Gut infection with *P. malariae* occurred between 60 and 90°F., the heaviest being at 70°F. No gland infection occurred with *P. malariae*.

In Calcutta the optimum conditions for the development of the parasites occur from November to March (Strickland *et al.*, 1933), whereas in Bombay the highest gland infection is noticed in the months of highest relative humidity, *i.e.*, from July to September. (Bentley, 1911).

A. philippinensis Ludlow.

Outside India it is distributed in Burma, Malaya and the Philippines and in India, Assam, Bengal and southern India.

It breeds mainly in tanks, borrow-pits and to a small degree in rice-fields. Krishnan (1940) pointed out that it is not particularly attracted by *Pistia*. According to Sen (1941) it shows a preference for *Spirogyra*, *Utricularia* and *Limnithemum*, *Pistia* standing low in the list of plants with which this species is found associated, though such association has been reported in the Philippines. The close relationship between *A. philippinensis* and certain aquatic plants has been referred to by Iyengar (1944).

It often shows a high sporozoite index and is essentially a domestic mosquito being found more in dwelling houses than in cowsheds.

The fact that it is a dangerous malaria carrier in Bengal (Sur, 1928 ; Bose, 1932 ; Iyengar, 1939, 1940), and is innocuous in Assam (Strickland, 1929 ; Ramsay, 1931) has been taken to indicate the presence of two physiologically different races.

A. annularis v.d. Wulp.

A. fuliginosus of earlier writers really comprised two species *A. annularis* and *A. philippinensis*. The difference between them has now been made clear by Christophers (1924).

It is widely distributed throughout the Oriental region.

It prefers stagnant water such as weedy tanks, rice-fields, swamps, lakes, borrow-pits, etc.

The adults are found particularly in cowsheds and they generally feed on cattle, its anthropophilic index being extremely low. Although infection has been recorded especially in Bengal, the infection rate is so low that it is considered to play an insignificant role in the transmission of malaria. Its importance in the Orissa coastal plains, where it is the principal carrier, has recently been pointed out by Sarathy (1932) and Senior White *et al.*, (1943). Although it has been found naturally infected in Yunnan it is not an important vector.

According to Christophers (1911), it can fly a distance greater than half-a-mile. This mosquito mostly feeds during the first part of night.

A. pallidus Theob.

In Central Provinces, where this species is found in large numbers, it is essentially a rice-field breeder. It also breeds in pools, tanks, lakes etc. in the presence of vegetation.

It rests in cowsheds.

Though infection has been reported from Bengal, Central Provinces, and other places, it is considered to be of only local importance.

A. maculatus Theob.

It is widely distributed in the Oriental Region and though it is a proved carrier in Malaya, direct proof of its acting as a vector in this country except in Shillong (Viswanathan *et al.*, 1941), has not been obtained. In India and elsewhere it occurs in the hilly regions.

It breeds in clear water in connection with hill streams exposed to sunlight. It is also found in seepages. It is very sensitive to organic pollution.

The adult rests usually in cow-sheds.

The artificial infectivity experiments of Strickland, Roy and Sen Gupta (1940) indicate that the Indian *A. maculatus* is not so susceptible to the local strain of malarial parasites as to that of Malaya. It has been found naturally infected in Yunnan (Robertson, 1941).

A. jeyporiensis James.

In India it is distributed in the eastern, central and southern parts and breeds in seepage pools, clear water streams, and in swamps.

The adults rest in the day time not only in cowsheds but also in human dwelling houses.

Salivary gland infection in *A. jeyporiensis* (type form) has not yet been found in nature though stomach infection has been recorded from the Jeypore Hills (Senior White, 1937) and from Mysore (Nursing *et al.*, 1934).

A varietal form, *candidiensis*, has been suspected as playing a considerable rôle in the causation of malaria in the Arakan Hills. Iyengar (1934) found gut infection in this species in Travancore. Both gut and gland infections have been discovered in this varietal form in Yunnan.

A. majidi Young and Majid.

It was at first thought to be a varietal form of *A. karwari* but has now been recognised as a valid species (Puri, 1928). It has been described from Bengal and Southern India. It probably takes no part in malaria transmission.

A. subpictus Grassi.

It is one of the most widely distributed *Anopheles* not only in India but also in the Oriental Region. It breeds in almost any type of still water, *e.g.*, in brick-pits, pools, drains, rice-fields, etc. It can tolerate a considerable amount of organic contamination of the water. It has been found breeding in association with *A. stephensi* in river beds and in cisterns. It is the predominating mosquito in the salt lake areas near Calcutta.

This species, though found in the neighbourhood of human dwellings, is a marked cattle feeder (Roy, 1943), and can be caught in large numbers in cattle houses.

Although gland infection has been recorded in this species, it is considered to play an insignificant role in malaria transmission in nature (Iyengar, 1931).

Mehta (1934) found that at 30°C, *A. subpictus* lives for 5 to 11 days. High humidity, about 90 per cent, is injurious to the life of the females of this species

and this is particularly marked at 25°C. At 40°C the females of *A. subpictus* do not survive for more than 24 to 50 hours when the humidity ranges from 50 to 90 per cent. The duration of life is comparatively enhanced when the temperature is lowered to 35°C. At this temperature and humidity varying from 30 to 90 per cent, the mosquitoes live from 3 to 8 days. At 25°C and 70 per cent relative humidity the adults may live even up to 20 days. Both low and high humidities are detrimental to the longevity of the females.

It has been reported as harbouring the parasites of bird malaria *Plasmodium precox* (Mayne, 1928). Russell (1931), on the other hand, reports that this mosquito would prefer death to feeding on canaries.

A. vagus Dön.

It is found mainly in the eastern and southern parts of India. The breeding habits are very similar to those of *A. subpictus*.

Though natural infection has been recorded in this species, it is not considered as a carrier of any importance in India.

The adults are caught in cow sheds ; they feed on cattle.

A. leucosphyrus Dön.

Though widely distributed in the Oriental Region, in India it is mainly confined to Assam, Peninsular India, and Ceylon. It is a jungle species and breeds in forest pools, and nullahs in partially shaded places.

Its importance as a malaria carrier is only local, e.g., in Digboi (Assam), where Clark and Chowdhury (1941) reported a high infection rate in this species ; the mosquitoes were all caught from human habitations and no cattle or goats were kept in that locality.

A. tessellatus Theob.

In India it is distributed in the east and south. It breeds in pools and though it has been infected in the laboratory, in nature it does not carry malaria.

A. aconitus Dön.

In India it is mainly confined to the eastern and southern parts. Outside India it has a wide distribution in Burma, Malaya, Indo-China, Java and Sumatra.

It breeds in grassy edges of irrigation canals and small streams and prefers to bite cattle rather than man in India, whereas in the Dutch East-Indies this species is more anthropophilic than zoophilic.

Though both gut and gland infections have been recorded in this country, it is considered, except in a few places, to be an unimportant species. In Chandpur (Bengal) Das (1943) found this species alone infected and thought it to be responsible for an outbreak of malaria. Similarly Senior White *et al.*, (1943) have incriminated it with malaria transmission at certain places on the Orissa coastal plain.

A. pulcherrimus Theob.

In India it has been recorded from the N.W.F. Province, Punjab, Sind and Gujerat. It is essentially a swamp breeder. Though it is a vicious biter, biting

both man and animals indiscriminately, only gut infection has been recorded in this species.

A. lindesayi Giles.

A large mosquito which has been recorded from altitudes of 4,000 ft. or over. It is principally a Himalayan species. It breeds in mountain streams.

A. gigas Swell. and Rodenwaldt.

This also is a large mosquito and has been recorded from high altitudes. It breeds in small pools along hill springs and is essentially a wild species.

A. umbrosus Theob.

A large and black mosquito and resembles *A. barbirostris* in general appearance. It is widely distributed in the jungle regions of Malaya where it is a carrier. In India it has been recorded only from Assam where it breeds in muddy pools containing rotten vegetation. It is a rare mosquito in this country.

A. barbirostris v.d. Wulp.

It is a large and black mosquito and is widely distributed in India. Though for all practical purposes it is regarded as a wild species, experimentally it can be infected with malarial parasites up to the oöcyst stage. It breeds in swamps, tanks etc., containing much decayed vegetation. The larvæ are found also in rice fields.

A. hyrcanus (var. *nigerrimus* Giles. and var. *sinensis* Weid.)

Two varieties of *A. hyrcanus* are known. The one found in India is represented by *nigerrimus* which is a cattle feeder and breeds in water polluted with faeces and urine of cattle and buffalo. It is also found in rice fields. From the malaria point of view it is totally innocuous. In Netherlands East Indies these two subspecies have not always been distinguished in reports on investigations on their ability to transmit malaria in nature. However, they have been experimentally infected with malaria in that country. Recently it has come to be considered a carrier of malaria of some importance under certain circumstances in Malaya (Hodgkin, 1933). It is the only vector in Formosa and Korea and in the plains in China. It is essentially a rice-field breeder.

A. kochi Dön.

This mosquito is mainly an Assam species and is a cattle feeder. It breeds in small pools and though it has been found naturally infected in the Dutch East Indies, no infection has been recorded in this species in India.

A. splendidus Koidzumi.

It is widely distributed in India but is never caught in large numbers at a time. Its malaria-carrying potentiality is probably nil. It breeds in small pools in the presence of aquatic vegetation.

A. ramsayi Covell.

Previous to 1927 it was confused with *A. jamesi*. In India it is found in the eastern areas and breeds in tanks and swamps in association with *Pistia*. Though

it has been found infected in nature, it is considered to play no rôle in the transmission of malaria. The adults are found in cowsheds.

A. turkhudi Liston.

This is essentially a cattle feeder and breeds in pools and in sluggish streams in the presence of aquatic vegetation. It has a limited distribution in India being found in Quetta and sometimes in Gujerat.

A. superpictus Grassi.

This is distributed from the Mediterranean region to the N. W. of India. Though it is an important carrier of malaria in Palestine, Greece and Mesopotamia, it has not yet been proved to play any rôle in malaria transmission in this country. It breeds in hilly river beds and prefers flowing water. It has a very limited distribution in India.

A. jamesi Theob.

Occurs in north Bengal, Assam, Southern India and in Ceylon. It breeds in ditches, ponds and tanks in the presence of aquatic vegetation. It rests principally in cowsheds and takes no part in the transmission of malaria.

A. theobaldi Giles.

Found mainly in the eastern and central parts of the Peninsula and breeds in shallow pools of clear water in the presence of green algae, also in rocky pools in connection with streams. Occasionally it is found breeding in wells.

A. karwari James.

Reported from Bengal, Assam, Ceylon, and the east coast of Madras. It breeds in clear water pools, ravines and seepage pools.

A. moghulensis Christ.

It was originally described by Christophers (1924) as a varietal form of *A. jeyporiensis*. It is principally distributed in the Central Provinces; it breeds in pools and nullahs. It is a wild species and the larvæ are found far away from human habitations.

A. culiciformis Cogill, *A. barianensis* James and *A. annandalei* Prashad.

All the above three are rare mosquitoes and all breed in tree-holes. *A. culiciformis* has been recorded only from the hills of the west coast of Peninsular India. *A. annandalei* is known from the eastern Himalayas, Khasi Hills and Ceylon. *A. barianensis* occurs in the Punjab and Kashmir at elevations of over 6,000 feet.

A. aitheni James.

This is essentially a jungle mosquito and is found in Assam. It breeds in ground pools and takes no part in malaria transmission.

A. dthali Patton.

It was previously known in this country as *A. rhodesiensis*, and described by Christophers and Khazan Chand.

It occurs in India in the hilly north western parts and in Dehra Dun and breeds principally in pools in hill streams. It has a limited distribution outside India, e.g., Baluchistan, Palestine etc.

Though it bites man freely it is not dangerous from the malaria point of view.

MALARIA.

As early as 1897 it was demonstrated that the *Anopheles* mosquito was the transmitter of malaria. The parasites of malaria live and multiply within the red cell and produce both asexual and sexual forms. The latter are called gametocytes and they remain within the blood corpuscles. The parasites are seldom found in the blood before 10 days after the discharge of sporozoites into the circulation by the feeding mosquito. When the gametocytes reach the alimentary canal of a suitable *Anopheles* host, the sporogony cycle begins. The parasites first make their appearance in the wall of the stomach especially in its posterior part, as oöcysts; these grow into sporoblasts and when they burst, sporozoites are liberated into the circulation. They penetrate the salivary glands of the mosquito, and are injected into the wound at the time of biting. It generally takes 4 days for the oöcyst to appear on the stomach wall and the glands become infected within 8-14 days. Environment, e.g., temperature and humidity, exercise a marked influence on the development of the parasites in the mosquito. To date there is no proof that malaria may be transmitted mechanically by insects or any other mosquito except by the genus *Anopheles*.

The oöcysts do not all mature at the same time; and as they mature and the cyst wall ruptures, fresh infection of the salivary glands takes place. The mosquito once infected retains the infection for a long time. This, however, depends on the frequency at which the sporozoites are liberated from the oöcyst. The number of oöcysts is also of importance in this respect.

With each feed sporozoites are discharged with the saliva, and so there is a natural tendency for the glands to become gradually depleted of infection.

It has now been conclusively proved that the development of oöcysts can be arrested for 2 or 3 weeks by cold and revived by appropriate warmth.

Bruce Mayne (1922) has shown that individual insects infected with the malaria parasite could be actually infective to man for 55 days, and could retain active sporozoites in the salivary glands for 70 days after the original infective meal.

Not all *Anopheles* are suitable hosts for the plasmodia nor are all those species which have been incriminated on either epidemiological or experimental grounds of importance in the transmission of malaria.

A mosquito should not be declared as a vector unless sporozoites have been detected in the salivary glands in the wild state as in many non-vectors the development of the parasite stops at the oöcyst stage and does not proceed any further.

FILARIAL AND OTHER DISEASES.

In addition to malaria it is possible that some anophelines are capable of conveying the larvae of *Wuchereria bancrofti*. Though *A. subpictus* has been reported

by Mayne (1928) to harbour the parasites of bird malaria, *Plasmodium precox*, it is not known to what extent *Anopheles* acts as a vector of this disease of birds. The aetiological agent in black-water fever is at present unknown. But there is no doubt that the disease is closely associated with repeated attacks of malaria and until this is disproved *Anopheles* must assume an indirect responsibility. Anophelines also take part in the transmission of the filaria parasites in dogs, *Dirofilaria immitis*.

THE USE OF INFECTED ANOPHELES IN THERAPEUTICS.

Infected Anophelines have been employed for the transfer of malaria to paretics. For some time it has been known that the high temperatures induced by malarial fevers have a therapeutic value in the treatment of paresis and certain other nervous disorders.

BLACK-SPORE.

During dissections of the stomach for malarial oöcysts one frequently encounters characteristic black bodies called black-spores which were first described by Ross (1898). These structures have been referred to in the literature by various names. They have been thought to have an association with the oöcyst of the malarial infected mosquito. The process of chitinisation may occur previous to the formation of sporozoites or when the cyst is full of sporozoites.

Their pleomorphic forms are a marked feature; some of the bodies being small and round, others spherical, flattened, banana-shaped, tangled skeins or sheaves of acicles. The shape is thought to be due to the manner in which the chitin has been deposited around oöcysts or sporozoites either in a single layer or in successive layers.

While a large majority of workers hold that the chitin bodies represent the products of death and degeneration of the malaria parasite, others believe that the black spores are nothing but the chitinous thickenings of tracheal tubes found in many insects and in mosquitoes other than *Anopheles* (Mayne, 1929). On the other hand, a more marked coincidence was noticed in the appearance of black-

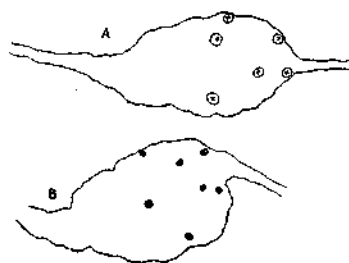


FIG. 35.
Diagrammatic representations of (A) Oöcysts showing extremely fine pigment granules, and (B) Black-spores in the stomach wall.

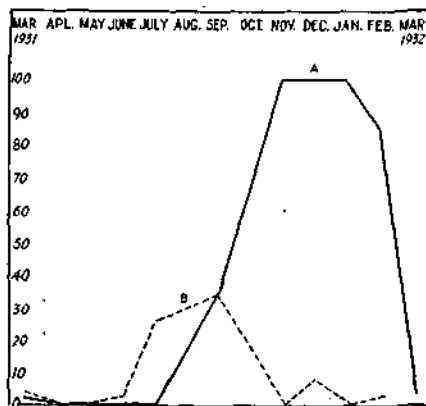


FIG. 36.
Malarial infection of the gut of *Anopheles stephensi* in Calcutta. (After Strickland and Roy).

A. Sporozoite rate; B. Zygotes showing chitinous bodies, rate %.

spores in the wall of the stomach of infected mosquitoes during the rainy season than at other times (Strickland and Roy, 1933).

A brief survey of the distribution of the principal malaria-carriers in the world is given below:

EUROPE

South coast of France (west of Marseilles near Port du Bouq and near Spanish border at Perpignan).	<i>A. maculipennis labranchiae</i> .
Portugal, West Spain and Holland	<i>A. maculipennis atroparvus</i>
South-East Spain	<i>A. maculipennis labranchiae</i>
Italy	<i>A. maculipennis labranchiae</i> (the most important)
	<i>A. maculipennis atroparvus</i> (along coast in northern Italy)
	<i>A. maculipennis messeae</i> (central parts)
Sardinia, Sicily, Corsica, Greece and Crete	<i>A. sacharovi (elutus)</i>
	<i>A. superpictus</i>
Albania	<i>A. superpictus</i> (mountain).
	<i>A. sacharovi</i> (coast)
Yugoslavia	<i>A. maculipennis atroparvus</i>
	<i>A. maculipennis labranchiae</i>
	<i>A. superpictus</i>
	<i>A. sacharovi (elutus)</i>
Rumania and Bulgaria	<i>A. maculipennis atroparvus</i>
	<i>A. sacharovi</i> (coast)
	<i>A. superpictus</i> (hills)
Cyprus	<i>A. claviger (bifurcatus)</i>
	<i>A. superpictus</i>
	<i>A. sacharovi (elutus)</i>
Russia	<i>A. maculipennis messeae</i>
	<i>A. maculipennis atroparvus</i>
Around Caspian Sea	<i>A. sacharovi (elutus)</i>

ASIA

Palestine	<i>A. claviger (urban)</i>
	<i>A. sacharovi (elutus)</i>
Mesopotamia	<i>A. superpictus</i>
	<i>A. stephensi</i>
Arabia	<i>A. gambiae</i>
Turkey	<i>A. sacharovi (elutus)</i>
	<i>A. superpictus</i>
Syria	<i>A. sacharovi (elutus)</i>
	<i>A. claviger (urban)</i>

Persia	<i>A. stephensi</i> <i>A. superpictus</i>
Iraq	<i>A. sacharovi (elutus)</i>
Mongolia and Turkestan	<i>A. superpictus</i> <i>A. maculipennis atroparvus</i> <i>A. sacharovi (elutus)</i>

AFRICA

North Africa	<i>A. maculipennis labbranchiæ</i> (along the coast of Morocco, Algiers and Tunis)
Egypt	<i>A. pharoensis (delta)</i>
Sudan	<i>A. pharoensis</i> <i>A. multicolor</i> (in brackish water in oasis)
Central and South Africa including Madagascar	<i>A. funestus</i> <i>A. gambiae</i>
Liberia	<i>A. gambiae</i> , <i>A. funestus</i> , <i>A. nili</i>
Uganda	In addition to <i>gambiae</i> and <i>funestus</i> minor rôle is played by <i>A. moucheti</i> and <i>A. pharoensis</i>

NORTH AMERICA

North America	<i>A. quadrimaculatus</i> (very widely distributed and the most important species) <i>A. crucians</i> in Virginia alone (not so important) <i>A. punctipennis</i> in North and South Carolina <i>A. maculipennis freeborni</i> in Pacific coast (interior)
Mexico	<i>A. quadrimaculatus</i> } North <i>A. crucians</i> } <i>A. pseudopunctipennis</i> in south and west. <i>A. albimanus</i> , Vera Cruz, Yucatan and Rio Grande River delta.

CENTRAL AMERICA

Guatemala	<i>A. albimanus</i> (the most important carrier) <i>A. argyritarsis</i> <i>A. pseudopunctipennis</i> <i>A. hectoris</i>
Panama	<i>A. albimanus</i> . Less important is <i>A. tarsimaculatus</i>
Other regions	<i>A. albimanus</i>

SOUTH AMERICA

A. albimanus is the most important carrier ; others like *A. albitarsis*, *A. argyritarsis* and *A. tarsimaculatus* also play considerable part as carriers.

Columbia	<i>A. albimanus</i> <i>A. argyritarsis</i> and <i>A. tarsimaculatus</i>
Venezuela	<i>A. albimanus</i> <i>A. argyritarsis</i> (coast) <i>A. albitarsis</i> and <i>A. darlingi</i> (plains) (epidemic malaria).
Brazil	<i>A. argyritarsis</i> and <i>A. albitarsis</i> . The former is particularly common in high lands of Rio de Janeiro where it is an important carrier and also in Sao Paulo. In the latter place <i>A. tarsimaculatus</i> is also a carrier ; in north western part <i>A. darlingi</i> . <i>A. gambiæ</i> has recently been introduced but its spread in Rio Grande do Norte and Ceara has been checked.
Guiana	<i>A. albimanus</i> <i>A. tarsimaculatus</i> <i>A. argyritarsis</i> (inland places)
Equador	<i>A. albimanus</i>
Argentina	<i>A. pseudopunctipennis</i> especially the north west <i>A. tarsimaculatus</i> (east coast), and in other places <i>A. albitarsis</i> , <i>A. argyritarsis</i>
Peru	<i>A. pseudopunctipennis</i>

AUSTRALIA AND OCEANIA

A. punctulatus is considered as a carrier on epidemiological grounds alone.

WEST INDIES

The most important carrier is *A. albimanus* ; of secondary importance is *A. tarsimaculatus*. *A. argyritarsis* plays the least important part.

CHINA

North	<i>A. pattoni</i> (It is strongly suspected)
Central	<i>A. hyrcanus</i> var. <i>sinensis</i>
South	<i>A. minimus</i> , <i>A. jeyporiensis</i> var. <i>candidiensis</i>

MANCHURIA

North	<i>A. maculipennis</i> var. <i>atroparvus</i>
West	<i>A. elutus</i>

KOREA AND FORMOSA

A. hyrcanus var. *sinensis*

JAPAN

A. hyrcanus var. *sinensis*

INDO-CHINA

A. minimus, *A. jeyporiensis* var.
candidiensis

MALAYA

A. maculatus (hills)
A. umbrosus (forests)
A. sundaicus (coast)

SIAM

A. sundaicus

DUTCH EAST INDIES

A. aconitus
A. sundaicus
A. maculatus

PHILIPPINES

A. minimus (*funestus-minimus* group)
A. sundaicus

NEW GUINEA

A. punctulatus var. *moluccensis*
A. bancrofti (north coast)

BURMA

A. sundaicus
A. minimus
A. philippinensis

ANDAMANS

A. sundaicus

CEYLON

A. culicifacies

INDIA

A. culicifacies
A. philippinensis
A. minimus
A. fluviatilis
A. varuna
A. sundaicus
A. stephensi

In determining the vector species in any locality the following points have to be taken into consideration.

(1) A sufficiently large number of dissections of all species have to be performed.

(2) The mere presence of oöcysts is without much significance unless infection of the salivary glands is also found. The accuracy of the oöcyst rate as a measure to test the suitability of *A. maculipennis* to transmit malaria holds as far as conditions in Europe are concerned (Swellengrebel and De Buck, 1931). In India where mosquitoes are known to behave in a diverse manner, often in contiguous localities, the oöcyst rate alone cannot be taken as an indication of the transmitting capacity of a mosquito unless the association of the particular species with malaria has already been proved.

(3) In order to act as a vector of major importance the infected species must be present in sufficient numbers during the time of malaria outbreaks and must have a span of life of at least 12 days.

(4) A large number of mosquitoes of that particular species should as a rule show infection in the salivary glands.

Regarding the susceptibility of different anophelines in this country it may be pointed out that

(a) *A. stephensi*, *A. minimus*, *A. fluviatilis*, *A. varuna* and *A. sundaicus* are particularly susceptible to infection and if they exist in sufficiently large numbers, they are extremely likely to prove harmful.

(b) *A. culicifacies* is a vector of major importance except in certain regions, e.g., Assam, Bengal and in limited areas in the south of the Peninsula.

(c) *A. aconitus*, *A. maculatus*, *A. annularis*, *A. subpictus*, and *A. jeyporiensis* var. *candidiensis*, although found infected in nature, have only local importance and some, e.g., *A. subpictus*, may be neglected altogether.

(d) In Assam *A. philippinensis* is harmless, whereas it is more or less the only infective species all over Bengal.

The following is a list of the important malaria-carrying anophelines in different localities in India:

Province		Principal vector or vectors	Subsidiary carriers which are of minor importance
Bengal	Calcutta (central)	<i>A. stephensi</i>	
	(eastern)	<i>A. sundaicus</i>	
	(environs)	<i>A. philippinensis</i>	<i>A. varuna</i>
	Coastal areas	<i>A. sundaicus</i>	<i>A. vagus</i> , <i>A. annularis</i>
	Deltaic Bengal	<i>A. philippinensis</i>	
Bihar	Dooars, N. Bengal	<i>A. minimus</i>	<i>A. pallidus</i> , <i>A. varuna</i>
	Singbhum	<i>A. fluviatilis</i>	<i>A. culicifacies</i>
		<i>A. varuna</i>	
		<i>A. minimus</i>	
	Ranchi Plateau	<i>A. fluviatilis</i>	<i>A. pallidus</i>
		<i>A. culicifacies</i>	<i>A. annularis</i>
	Udaipur State	<i>A. culicifacies</i>	<i>A. fluviatilis</i>
		<i>A. pallidus</i>	
	Darbhangha	<i>A. culicifacies</i>	
	Purnea	<i>A. philippinensis</i>	
Assam	The whole of Assam	<i>A. minimus</i>	<i>A. aconitus</i>
			<i>A. annularis</i>
			<i>A. philippinensis</i>
			<i>A. ramsayi</i>
	Digboi	<i>A. leucosphyrus</i>	
	Lumding	<i>A. culicifacies</i>	
	Shillong	<i>A. maculatus</i>	
		<i>A. annularis</i>	
	Lakhimpur and Gouripur	<i>A. minimus</i>	

Province	Locality	Principal vector or vectors	Subsidiary carriers which are of minor importance.
<i>Arakan</i>		<i>A. jeyporiensis</i> var. <i>candidiensis</i>	
<i>Madras</i>	Ennore-Nellore coastal area	<i>A. culicifacies</i>	
	Tanjore	<i>A. culicifacies</i>	
	Wynaad	<i>A. fluviatilis</i>	
	Travancore	<i>A. fluviatilis</i>	<i>A. culicifacies</i>
		<i>A. varuna</i>	<i>A. jeyporiensis</i> var. <i>candidiensis</i>
	Eastern Ghats. (Jeypore Hills)	<i>A. fluviatilis</i>	<i>A. culicifacies</i>
		<i>A. minimus</i>	<i>A. aconitus</i>
		<i>A. varuna</i>	<i>A. jeyporiensis</i>
<i>Mysore</i>	Mysore city	<i>A. stephensi</i>	
	Kolar	<i>A. culicifacies</i>	
		<i>A. fluviatilis</i>	<i>A. varuna</i>
		<i>A. minimus</i>	
	Hiriyur area	<i>A. culicifacies</i>	<i>A. stephensi</i>
		<i>A. fluviatilis</i>	
	Mandya area	<i>A. culicifacies</i>	
	Gargeshwari area	<i>A. culicifacies</i>	<i>A. aconitus</i>
			<i>A. fluviatilis</i>
	Nagenhalli area	<i>A. culicifacies</i>	<i>A. varuna</i>
		<i>A. fluviatilis</i>	
	Mudigere area	<i>A. fluviatilis</i>	<i>A. jeyporiensis</i>
			<i>A. culicifacies</i>
	Nilgiris	<i>A. fluviatilis</i>	
<i>Bangalore</i>	Bangalore city	<i>A. culicifacies</i>	
		<i>A. stephensi</i>	
<i>Hyderabad State</i>	Nizamabad	<i>A. fluviatilis</i>	
<i>Orissa</i>	Chilka Lake	<i>A. sundaicus</i>	
	Puri	<i>A. sundaicus</i>	<i>A. annularis</i>
	Coastal plain	<i>A. annularis</i>	
		<i>A. aconitus</i>	
<i>Bombay</i>	Bombay city	<i>A. stephensi</i>	
	Poona	<i>A. culicifacies</i>	
	North Kanara	<i>A. fluviatilis</i>	
	Western Ghats (Anaimallai Hills)	<i>A. fluviatilis</i>	

Province		Principal vector or vectors	Subsidiary carriers which are of minor importance.
<i>Central Provinces</i>	East Satpura range	<i>A. fluviatilis</i>	<i>A. culicifacies</i>
		<i>A. varuna</i>	<i>A. annularis</i>
<i>Baluchistan</i>	Bhopal	<i>A. culicifacies</i>	
	Quetta	<i>A. superpictus</i>	<i>A. stephensi</i>
<i>Kutch State</i>		<i>A. culicifacies</i>	
		<i>A. stephensi</i>	
<i>Sind</i>	Hyderabad (Deccan)	<i>A. stephensi</i>	
	Sind, both upper and lower	<i>A. culicifacies</i>	
<i>Patiala State</i>		<i>A. culicifacies</i>	
		<i>A. fluviatilis</i>	
<i>U. P.</i>	Lucknow	<i>A. stephensi</i>	
	United Provinces as a whole	<i>A. culicifacies</i>	
<i>Punjab</i>	Punjab as a whole	<i>A. culicifacies</i>	
<i>Rajputana</i>	Ajmere city	<i>A. culicifacies</i>	
<i>Ceylon</i>		<i>A. culicifacies</i>	

CERTAIN FACTORS CONNECTED WITH THE SPREAD OF MALARIA

Drainage and Malaria.

Whenever the natural flow of water in any locality is obstructed either by embankments, silting up of river beds or by excavation of earth for building roads, railways, huts, etc, it creates conditions favourable for mosquito breeding. Among them borrow-pits are extremely harmful for they hold water for a sufficiently long period especially during the time when temperature and humidity are most favourable for malaria transmission.

It is therefore apparent that the spread of malaria in any locality can be traced to an increase in the activities of man in the direction of his altering the natural topography of the land by real or so-called engineering projects, which can be both avoided and quickly remedied.

Irrigation and Malaria.

The close association between malaria and the present system of irrigation has long been known. Although irrigation has generally brought agricultural benefits to the people, it has also brought disadvantages in the way of greatly increasing

the incidence of malaria in areas which were previously healthy. The high incidence of malaria and its persistence in irrigated localities have been pointed out by Sinton (1930), Covell and Baily (1936) and Russell (1938).

It is, however, not directly due to the irrigation itself but to the adoption of a defective system. The different ways by which irrigation may bring malaria are:—

(1) It creates conditions extremely favourable for the breeding of *A. culicifacies*, due to leaks, seepages, improper delivery of water, etc.

(2) It causes the subsoil water level to rise.

(3) The humidity is increased at a time when the atmosphere is normally dry.

Water hyacinth and Anopheline breeding.

Eichornia crassipes, the common water hyacinth, is a native of Brazil but it has now become well established all over the globe. Its cultivation was once advocated for the control of mosquitoes especially of anopheline larvæ with the idea that by its rapid growth it would prevent mosquito breeding. No biological factor peculiar to this plant and unfavourable to anophelines has been discovered by Barber and Hayne (1925) and Sen (1941), and it is thought that its cultivation will prove of little value. In regions where it has overgrown, extensive production of anophelines is seldom noticed and species such as *A. annularis*, *A. hyrcanus*, *A. barbirostris* and *A. aconitus* are generally found in association with this plant (Strickland and Chowdhury, 1927).

Rice cultivation and Malaria.

It is widely believed that rice cultivation and malaria go hand in hand. Although there may be an abundance of anopheline larvæ, the dangerous species generally prevail in small numbers in paddy fields. In Bengal, Sen (1935) failed to find any correlation between the malarial intensity of a locality and rice cultivation on account of the fact that *A. philippinensis*, the principal vector, breeds in small numbers in rice fields. This lack of correlation has also been stressed by other workers elsewhere. However, larvæ of *A. culicifacies* have been reported as breeding in newly wetted fields prior to ploughing and also in recently planted rice-fields in certain parts of Southern India; the breeding generally ceases when the plants attain a height of 12 inches from the surface of the water (Russell and Rao, 1940).

Rice cultivation is really not a factor of any importance in favouring the occurrence of endemic malaria. Malaria surveys have shown that rice cultivation in the vicinity of towns is not always associated with a spleen rate of appreciable proportions, and it thus appears that it is not the cultivation of rice alone, but rather circumstances associated with it, that supply the environmental conditions favourable to endemic malaria. (Gill, 1930).

Houses and Malaria.

Malaria in Europe, North America and Brazil is considered to be a house disease, most of the malaria being transmitted in the house by a few house-loving, long-lived female *Anopheles*. There is at times a condition of gonotrophic dissociation when the need to oviposit ceases, while the need to feed does not. Mosquitoes

in this condition remain in the house where they have fed and are capable of becoming malarious and of transmitting malaria. The kind of blood meal consumed by the mosquito caught in houses shows that they are not tied to any particular feeding place and an *Anopheles* caught in the house has often had her previous meal from some animal outside it. The above represents the views expressed by the League of Nations Malaria Committee and it is fairly clear that malaria in the above countries is contracted within the house, and hence house-catching of resting anophelines in those countries has been exalted to an important place in methods of malaria control.

Whatever the validity of this theory in regions where anophelines remain in houses after biting, it can have no reference to the major malaria problem of this country where the mosquito has a tendency to leave the room soon after feeding.

It is no doubt probably true that the locus of infection by *A. gambiæ* in Africa, and by *funestus* group of mosquitoes representing, *A. minimus*, and *A. fluviatilis* and *A. varuna*, in India, is the dwelling house or cattle shed or their immediate neighbourhood. It is for this reason that spray killing of adult *Anopheles* has been so strongly recommended. Its importance was also recognised by Le Prince (1926) who advocated the destruction of gorged female *Anopheles* mosquitoes at a definite hour each morning. After the mosquitoes become engorged they rest relatively close to where they obtained their blood meal.

THE GENESIS OF MALARIA EPIDEMICS

As was first observed by Christophers (1911) in the Punjab, an epidemic of malaria always follows an excessive rainfall resulting in an abnormal increase of the carrier species, *A. culicifacies*, within a short time. The most striking feature of the atmospheric conditions is the relatively high degree of humidity prevailing during the pre-epidemic period. The maintenance of high atmospheric humidity in association with high temperature causes an increase in the numerical prevalence, longevity and metabolic activity of the insect carrier, and rapid completion of the sexual cycle of the malaria parasite in the mosquito host. It has also been suggested that the sudden onset of these climatic conditions may possibly exercise an indirect influence on the malaria parasite in the human host, so that an increased number of parasites become available for transmission (Gill, 1928).

Covell and Baily (1932) lay particular stress on the increase in the length of the period of high, sustained, relative atmospheric humidity as the main factor in the production of malaria. While the normal period of transmission in Sind, according to these authors, is not more than 6 weeks, they showed that it may be increased by about 4 weeks in years of excessive rainfall or flooding during the monsoon period. In addition to excessive rainfall, the other associate factor, the lowered communal immunity, is also an important contributory cause.

In the beginning of the epidemic there is a very high parasitic rate but the spleen rate is low. Though there is a paucity of gametocyte carriers among the population in the pre-epidemic period, there is usually a great increase within a month from the time the anopheline has ingested an infective feed. The parasite rate generally reaches its acme about the 12th week from the commencement

of the epidemic. The post-epidemic period is characterised during the first two years following the epidemic by enhanced waves of morbidity in the spring and autumn, while the birth rate in the year succeeding the epidemic is abnormally low.

In the epidemics in the Punjab there is a great increase in the malignant tertian parasite rate, followed by an increase of benign tertian infections during the months immediately succeeding an epidemic. This may possibly be due either to fresh infection with *P. vivax*, to delayed infection, or to late relapses. It is now known that cases of malaria occurring in Holland in the spring are the result of primary infection acquired during the previous autumn (Schöffner, Kortweg and Swellengrebel, 1929). It is for this reason that the infection of mosquitoes is practically absent during the spring when malaria is rife (Swellengrebel, 1924). Sinton (1931) also recorded a considerable number of latent primary infections with *P. vivax*.

The occurrence of late relapses between 30 and 40 weeks after the time when the primary infections were received has been pointed out by Anderson (1922) and James (1931).

In the great malaria epidemic in Ceylon transmission also was due to *A. culicifacies*. While epidemics in the Punjab follow excessive rainfall during the monsoon, the Ceylon epidemic had its origin in the drying up of the rivers with the formation of shallow pools of clear water. These provided ideal conditions for *culicifacies*, and the rains in October filled borrow-pits, quarry pools, and other potential breeding places. Thus conditions favourable for an enormous multiplication of the mosquito were provided. High infection rates, even 21 per cent. among the mosquitoes caught inside the houses, have been recorded.

With the single exception that the benign tertian parasite apparently played a greater part in the Ceylon epidemic, its general feature was the same as that noticed in the Punjab and Sind.

MALARIA AND MALARIA-MOSQUITO SURVEY

A considerable amount of knowledge has accumulated regarding the habits and bionomics of mosquitoes. It has now been fully realised that the dangerous ones are those that bite man and among them many are able to carry diseases. It is therefore logical to hold that attention should be concentrated on the eradication or control of these mosquitoes alone instead of an indiscriminate attack against all. In the beginning Ross advocated antimosquito measures against all mosquitoes prevailing in the locality with the idea that not only malaria but all mosquito-borne diseases would thereby be controlled. Such assault against mosquitoes in general cannot, however, be carried out in practice owing to the fact that it would not be financially feasible. It has been demonstrated by Watson in Malaya that it is not only possible but is also economical to eradicate or greatly reduce the incidence of malaria in a locality by adopting measures against the proved carriers only.

In undertaking species control it is necessary to be provided with adequate knowledge of (a) the species that are dangerous, (b) their distribution and prevalence, (c) their life history and habits, and (d) of other factors pertaining

to malaria in that particular locality. These constitute a malaria survey which should not only precede but also accompany antimosquito measures. In order to judge the progress of antimalaria operations, quantitative estimation of the incidence of the disease is also necessary. The various factors involved in malaria survey are dealt with below.

(a) Collection of anopheline larvæ. (b) Collection of adult anophelines. (c) The study of the habits of the mosquitoes. (d) Dissection of mosquitoes. (e) Detection of infection in mosquitoes. (f) Spleen-index of children. (g) Parasite index. (h) Transmission season. (i) Laboratory infectivity experiments. (j) Infective

density of anophelines. (k) Accessory factors, *e.g.*, meteorological conditions, general configuration of the country, economic conditions of the local inhabitants, birth rate, death rate, etc.

Before the actual work of the survey is undertaken it is necessary to prepare a sketch map of the locality up to an area of half-a-mile from the outer perimeter of the area to be controlled and the above data as far as possible should be plotted on the map. The malaria survey should also be extended to that area which is supposed to cover the distance of flight of anopheline mosquitoes in general.

COLLECTION OF ANOPHELINE LARVÆ

Bearing in mind that *Anopheles* mosquitoes may breed in all types of water such as in pools, tanks, ditches, borrow-pits, cisterns, drains, wells, cattle hoof-marks, grassy-edged streams, irrigation channels, river beds, rain-water puddles, and in puddles in hill streams etc., it is essential that all water collections, large and small, clean or dirty, should be properly searched. The larvæ are collected from the edges by means of an ordinary enamel fry pan, against the white background of which the mosquito larvæ show up very well. They are transferred by a wide-mouthed pipette to a collecting bottle containing some water and a few aquatic plants from the same source as the larvæ. Any handy

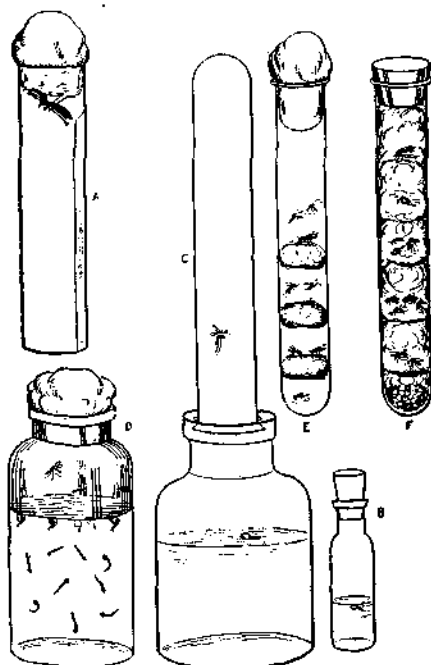


FIG. 37.

A, A glass tube lined with filter paper and well soaked in water is used for inducing mosquitoes to lay their eggs in the laboratory. As far as possible blood-fed wild females should be used for this purpose.

B & C, For collecting larval skin, mature larvæ must be reared separately. The larval skin is preserved in spirit (B), and the pupa is bred out into an adult (C).

D, A wide-mouthed bottle is generally used for breeding a large number of adult mosquitoes from larvæ.

E, More than one living adult mosquito can be captured in a single tube.

F, Mosquitoes may be preserved if necessary in corked tubes. At the bottom a few bits of naphthalene are placed; these will prevent other insects, which habitually prey on such preserved specimens, from entering the tube.

wide-mouthed bottle will serve as a collecting bottle. Quinine bottles are very commonly used for this purpose. Each bottle should be properly numbered and all records in regard to the breeding habits of different batches of larvæ are to be entered separately in the book.

Larvæ from wells may be collected with a properly devised net. When larvæ are suspected of breeding some distance from the edge, it may be necessary to affix the fry pan to the end of a long handle.

When the larvæ have been brought to the laboratory, they should be sorted out. Only a few mature larvæ are to be bred out separately in wide-mouthed bottles, the open end being closed with cotton wool. Examination must be made daily. The dead larvæ and pupæ together with larval skins are to be preserved in 80 per cent. spirit. Immature larvæ, whether dead or living, must also be preserved in the same way. A point to remember is that when larvæ are crowded in a small place and when adequate food is lacking, they become cannibalistic. It may be necessary to change the water from time to time and as far as possible natural water should be used.

When the adult emerges it is collected in a test-tube, the open end of which is placed against the open end of the bottle. It is advisable to let the adult die a natural death in the test tube rather than kill it with chloroform. The mosquito then dies with its wings and legs fully stretched.

COLLECTION OF ADULT ANOPHELES

(i) For collecting adult mosquitoes, dwelling houses, thatched huts, culverts, cattle sheds, pig styes, cellars, etc., are to be searched. The mosquitoes generally take shelter in dark places. They often sit on thatch or on strings and soots hanging from the roof. Search should be made if possible twice, once in the morning and again in the evening, otherwise in the morning only. An electric torch will be of invaluable assistance. The usual practice is to place the open mouth of a test tube over the resting mosquito. In this way mosquitoes may be

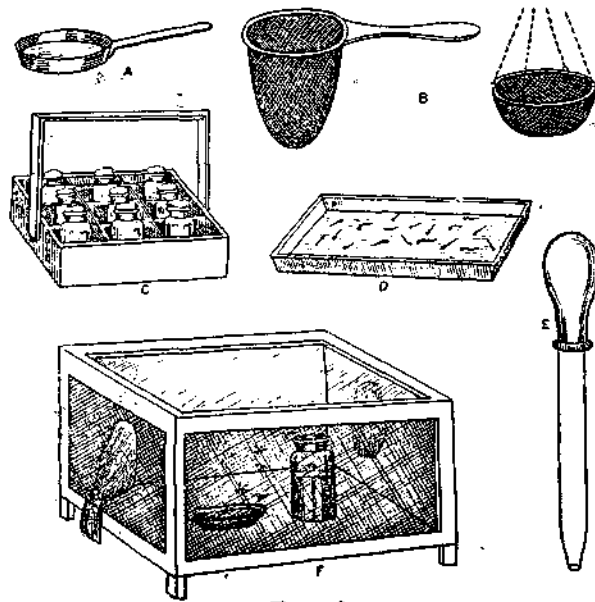


FIG. 38.

Laboratory equipment for mosquito and malaria work. A. Ordinary fry pan. B. Net for catching larvæ. C. Convenient way of carrying larvæ in bottles. D. Tray. E. Pipette for picking out larvæ from tray. F. A glass topped cage: used for (a) keeping adult mosquitoes alive on raisin and water, (b) for breeding adults from mature larvæ or pupæ, and also for (c) feeding mosquitoes on the blood of volunteers.

separately enclosed in different compartments of the same tube by pushing a cotton-wool plug as far down as possible after a mosquito has been captured.

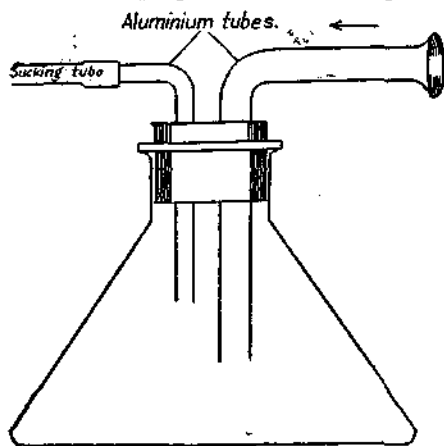


FIG. 39.
Suction apparatus.

(ii) When the mosquitoes are out of reach, they may be caught with a small suction tube. This is an ordinary tube, the open end being so designed as to allow the mosquito to run in but it cannot escape; to the other end is attached a rubber tube for suction.

(iii) The upside down umbrella in collecting mosquitoes in thatched ceilings is a modification by Barber and Rice (1938) of the well known procedure by which mosquitoes killed or stupefied in a room by various sprays are collected on a sheet spread over the floor. The mosquitoes are killed or

stupefied by direct hit with pyrethrum spray; these fall on an umbrella held with the bottom side up under the thatch and in close contact with it. The umbrella should be specially made with white cloth and with a considerably smaller number of gadgets than usual.

HABITS OF MOSQUITOES

Considerable information of the habits of different mosquitoes particularly in regard to their preference for human or cattle blood, the time of their feed, their daytime resting places, the time of their migration into dwelling houses at night, their breeding habits etc. may always be gathered from the behaviour of the adults. Such information will be of invaluable help not only in evolving the best means of control measures but also in judging the progress of antimosquito operations.

DISSECTION OF MOSQUITOES

Salivary glands.

The object of dissection of an *Anopheles* mosquito is to study the sites of development of malarial parasites in its body. These are the salivary glands and the stomach.

Dead and dried mosquitoes are quite unsuitable for dissection. Fresh ones should be killed with chloroform.

As far as possible sufficient time must be allowed to pass for the digestion of the blood meal otherwise the detection of oöcysts in the stomach containing fresh blood becomes a little difficult. Such mosquitoes may be sustained on raisins and water for two or three days. Dissection for salivary glands is better practised in females than in males as the organs are better developed in the former and it is easier to expose the glands. The mosquito should first be identified with the help of a synoptic table; the wings and legs are next removed. In order to eliminate the surface tension it is necessary to immerse the insect in rectified

spirit for 10 seconds in a tube and to shake it. Mosquitoes when kept in spirit for a long time are liable to become hard. It is next immersed in normal salt solution in a watch-glass. Dissection is best done with needles under a dissecting microscope against a dark back ground. The insect is placed on a glass slide in a drop of salt solution with either the head pointing towards the

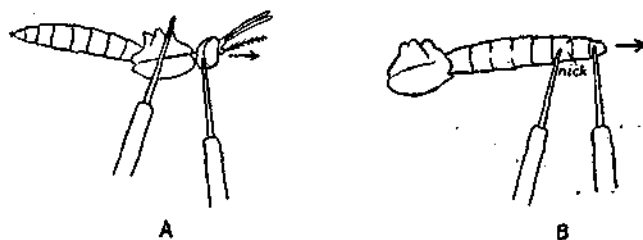


FIG. 40.
Dissection of (A) salivary glands and (B) alimentary canal of a mosquito.

right and its back towards the dissector or the head pointed towards the dissector and abdomen away from him. A blunt needle held by the left hand is placed across the thorax in order to steady the insect and with another needle held in the right hand and placed on the back of the head, gentle traction is exerted on the head away from the body along the axis of the abdomen; simultaneously gentle pressure on the thorax with the other needle will cause the glands to bulge forward. When the head is carefully separated from the body in the way stated above, the glands will be found projecting out of the tissues at the cut end of the neck. The glands are easily recognised under the low power of the microscope by their glandular structure, and their transparent glistening appearance. The glands are now separated from the neck tissue using very clean needles. They may now be covered with a cover glass, the edges of which are ringed with vaseline to prevent desiccation.

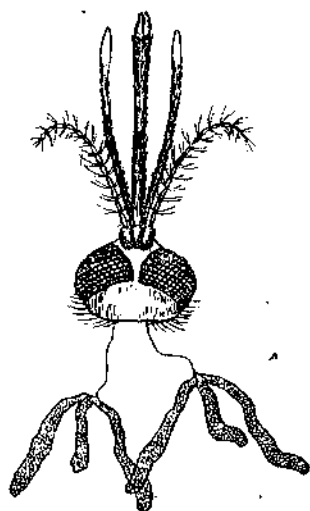


FIG. 41.
Salivary glands of a mosquito attached to the head.

The glands of either side consist of three acini, the ducts of which join soon after leaving the acini to form a single common duct.

Oesophageal diverticulæ are liable to be confused with salivary glands. They always contain bubbles of gas whereas the latter are glistening, highly refractile bodies, and have a uniform glandular appearance.

Dissection of oesophageal diverticulæ.

The dissection of the diverticulæ is difficult. The best way to isolate them is by the anterior method, because by the posterior route the sacs nearly always break.

The first step is the separation of the dorsal shield, *i.e.*, the chitin and the muscles of the dorsum of the thorax, care being taken not to injure the underlying tissues. There is considerable pressure inside the sacs, which cannot suffer much greater distension than that already existing and even a little

pressure with the needle is likely to press out some of the gas bubbles. Success therefore greatly depends on a good and complete separation of the scutum of the thorax. The sternite and the pleura are likewise carefully removed. The chitin of each segment of the abdomen is then split open. The abdomen is cut transversely at the lower level of the ventral sac. The tissues surrounding the gut and the diverticula are then gently separated.

Alimentary canal.

After the salivary glands have been dissected, the same mosquito may be utilised for dissection of the alimentary canal. The mosquito is placed in a drop of salt solution on the slide and two lateral cuts involving the cuticle only are made with a needle between the 7th and 8th abdominal segments taking care not to injure the alimentary canal. The soft tissues may be pressed by a blunt needle to the opposite side before the cuticle is cut. The insect is to be kept steady by placing one needle across the thorax and with the other needle the last segment is drawn away from the body by making steady traction. As this segment is pulled away, the alimentary tract and the reproductive system which are attached to the hind end of the body, will gradually come into view. In this way the entire alimentary system can be exposed.

DETECTION OF INFECTION IN THE MOSQUITO

(a) *Oöcysts in the wall of the stomach.* These generally appear on the 4th day after the infective blood feed. They are round, cannot be detached from the gut wall by rolling or by flooding, and are placed within the wall of the stomach. They moreover contain distinct pigment granules. They do not take ordinary stains, e.g., eosin, but are easily stained with methylene blue. When mature they contain a very large number of sporozoites; these escape when the oöcyst is ruptured.

The oöcyst stage of the different plasmodial parasites can be identified without much difficulty by noting the character and arrangement of the pigment. In *P. vivax* grains of pigment are numerous (more than 30 or 40), lighter brown than in any other species, their rods and specks scattered throughout the parasite in a definite pattern. The pigment grains in *P. malariae* are numerous, but larger, thicker and much darker than *P. vivax*. In shape they are more like cocci than rods or specks and tend to be bunched together in a clump round the contour of the cyst. The contour of the oöcyst is remarkably well defined in *P. falciparum*. Grains of pigment are few about 10 or 12, large in size and black. They are characteristically arranged as a streak across the oöcyst or as a half circle. In *P. ovale* the grains are more like those of *P. malariae*. They are always arranged in one or other of several definite patterns of which the most common is two rows of dots crossing each other at right angles.

Preservation and staining of oöcyst.

(1) Pass Bouin's fixative between the slide and the cover glass. After 15 minutes carefully lift the cover glass, pass the stomach through 70%, 50% and 30% alcohol allowing 5 minutes for each treatment and thereafter leave the object in distilled water for at least one hour, preferably two. It is then stained

in Mayer's acid hæmalum for at least 2 hours, preferably the object should be left in the stain overnight. Then wash in water, pass through ascending grades of alcohol 30, 50, 70, 80, 90, absolute alcohol, alcohol and xylol, clove oil, and mount in Canada balsam.

(2) Place the dissected stomach in a mixture of half formalin and half glycerine for 24 hours; then in glycerine for 24 hours; change and mount in pure glycerine. The margin of the cover-glass may be ringed with coloured wax and thereafter with 95 per cent solution of wax in alcohol.

(3) Pour a few drops of saturated solution of picric acid in water and leave the specimen in this solution for 15 minutes when the stomach is likely to adhere to the slide. Leave in distilled water overnight; stain with Mayer's hæmalum, then wash in running water. For dehydration, xylol and carbolic acid may be used. Mount in Canada balsam.

(4) An infested stomach may be preserved in pure formalin indefinitely. Before mounting, it should be washed in water, stained for about six minutes with hæmalum, washed in a current of water and mounted on a slide in the usual way. The stomach after washing in water may be passed through ascending grades of alcohol from 30% to 80% and then mounted permanently in Berles's fluid.

(5) A fresh stomach preparation should be flattened out by pressing with a cover glass; the latter is now removed and the salt solution should be allowed to dry up. As soon as the drying is nearly complete, pour on one or two drops of Bless's fluid or Bouin's fixative and allow the fixative to act for 5 minutes. The slide is then reflooded with the fixing fluid and left inside a Petri dish for 1 hour. The preparation is washed in water. Next stain with Delafield's hæmatoxylin overnight. The specimen will look dark. It should be carefully washed in tap water and examined under the microscope to find out the depth of the staining. Excessive staining is removed by acid alcohol used well diluted and the process watched under the microscope. As soon as the necessary depth of staining is reached, *i.e.*, when the oöcysts stand out prominently and the granules inside them are distinctly visible, wash off the alcohol with water; pass through ascending grades of alcohol beginning with 30% and ending with absolute alcohol; clear in xylol and mount in Canada balsam.

(6) Iron-hæmatoxylin stain. The usual procedure as for protozoological objects is followed.

(7) Rapid method. (Green, 1932).

This has certain advantages: it saves considerable time during fixation, staining and mounting of the specimen, also in permitting pigment within oöcysts being seen on any part of the midgut.

(1 volume of formalin is taken and made up to 36 or 38 volumes by adding distilled water. To every 100 c.c. of this solution is added 0.859 gm. sodium chloride).

(a) 1 per cent formaldehyde in normal saline is run under the coverslip and the excess of fluid removed with small strips of blotting paper until a suitable degree of flattening is obtained. The slide is then put aside for about 10 minutes in a closed Petri dish containing moist blotting paper.

(b) Pugh's stain (19 c.c. of toluidine blue in 20 c.c. absolute alcohol ; and 20 c.c. glacial acetic acid and sufficient distilled water to 1,000 c.c.) is then run under the cover slip, and, if the staining of the specimen is watched under a dissecting microscope, the midgut will be seen to have adhered, as a rule to the slide or the cover slip, usually the latter. The stain is allowed to act for about 5 minutes.

(c) The coverslip is gently floated to one side and removed. The specimen on the slide or coverslip is then dipped carefully into the following mixtures kept in closed jars: (i) acetone 95 parts and xylol 5 parts, for 30 seconds ; (ii) acetone 70 parts and xylol 30 parts, for 1 minute ; (iii) acetone 30 plus xylol 70 parts, for 1½ minutes ; (iv) pure xylol for 2 minutes.

(d) Xylol is removed by blotting paper and a drop of clove oil is placed on the specimen. After the excess of clove oil has been removed it is mounted in Canada balsam or Euparal.

(b) *Detection of sporozoites in the salivary glands.*

The detection of sporozoites in the salivary glands is easy when they are present in large numbers. A heavy infection of the glands is a very common feature

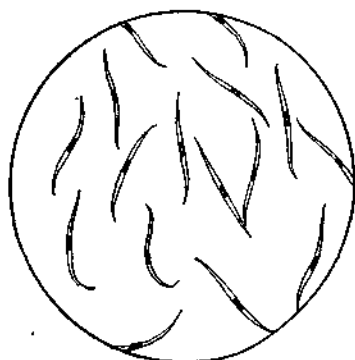


FIG. 42.
Sporozoites stained with Giemsa's stain as seen with oil immersion lens.

unless the mosquito has become old and the infection is dying off due to continued loss of sporozoites with each feed. As soon as the glands are ruptured by pressure the sporozoites escape in streams from inside the gland. When examined under ½ inch lens they appear as thin, curved rods and have a characteristic slow movement, on account of which they do not always maintain a uniform shape. Under unfavourable conditions the movements of sporozoites may become sluggish. (Boyd and Stratman-Thomas, 1934 ; Barber, 1936). In size they are twice the diameter of a red blood corpuscle.

Dissection of salivary glands may be done in a solution of 0.5 per cent brilliant cresyl blue in physiological salt solution (Giovannola, 1934) ; this will stain the living sporozoites which will aid the diagnosis of infection in the mosquito.

Sergents Method of detection of infection in the mosquito.

The sporozoites after their liberation from the oöcysts into the hæmocoel or the "body-cavity" of the mosquito invade all the organs of the body except the ovaries, particularly the musculature of the palps and scutellum which become crammed with parasites, at least for some time (Mayer, 1921; Mühlens, 1921). This has been practically utilised in evolving an easier and much quicker method for the detection of sporozoite infection in the body of the mosquito. This is known as Sergents' method (1910) and has been reported upon by Soesilo (1928) and also by Strickland and Roy (1931) as quite reliable for adoption in field malariology. By this method 361 individuals per diem were dealt with by Strickland and Chowdhury (1933).

After the avulsion of the legs and wings, the mosquito is placed on a glass slide and the head decapitated by means of a sharp-edged needle. A blunt needle is then placed across the thorax, and gentle pressure is applied, so that some "body fluid" mixed with the juice from the crushed salivary glands exudes through the cut end of the neck into a minute drop of normal saline, previously placed upon the slide; the smaller the drop of saline into which the hæmocoelæ fluid escapes, the more quickly one finds the sporozoites, for a smaller area has to be searched. A cover-glass is then applied and the preparation examined under $\frac{1}{4}$ th inch objective with No. 8 ocular. The sporozoites in fresh specimens are either straight, a little curved, sickle or S-shaped, and are feebly motile.

Staining of sporozoites and salivary glands.

(a) The slide containing sporozoites in salt solution should be dried in air and then fixed in Merck's methyl alcohol for 10 minutes. When nearly dry, the sporozoites are stained with Giemsa's; the cytoplasm stains blue and the nucleus red. The stain fades away within a short time.

(b) The salivary glands should be fixed with Bless's fluid for 24 hours and left in Delafield's hæmatoxylin overnight. Differentiation is done with weak, acid-alcohol. It is then dehydrated in alcohol and mounted in Canada balsam.

MEASUREMENT OF ENLARGED SPLEEN

Different methods have been used. Examination for splenic measurements must be made in the erect position. The spleen rate unless otherwise stated is meant to indicate the percentage of children aged from 2 to 10 years with enlarged spleen.

(a) By finger-breadth: this notation is the one most commonly used and for all practical purposes it gives us the necessary information. A finger breadth may be taken as 2 cms.

(b) *Average enlarged spleen and average spleen.* Mark the apex of the spleen below the costal margin and then measure (i) the distance in centimetres between this mark and the umbilicus, (ii) the distance between the mark and the costal margin.

If the apex is below a horizontal line passing through the umbilicus, the measurement is to be recorded as "minus".

For the purposes of field work the *average enlarged spleen* can be sufficiently indicated by taking the mean value for (i), i.e., all the positive figures for the apex-umbilicus measurement are to be added from which the sum of the minus figures is to be subtracted. The result is to be divided by the number of children whose splenic measurements are recorded. Children without palpable spleens are not counted in this process.

The *average spleen* is obtained by dividing the sum of all (iii) measurements by the total number of children examined, instead of only by the number with recorded splenic enlargement.

In the case of (i) and (ii) the smaller the measurement, the larger the spleen. The degree of the endemicity of malaria in any locality is generally determined by the spleen index. Thus hyperendemic conditions are defined as those in which

Key to the identification of Anopheles (females only) of India.

Group I.

Costa uniformly dark, i.e., not interrupted by any pale spot;
also no white spot on the wing field.

Anterior forked cell much larger than
the posterior.

A. aitheni.

Anterior forked cell of nearly the same
size as the posterior.

Distinct white banding at the
distal end of hind femur.
Frontal white scale
present.

A. barianensis.

Banding absent.
absent.

A. culiciformis.

Scale
tuft

The following are some common methods of staining blood for the detection of malarial parasites:—

For thin films:

(i) Leishman's method is well known. It contains methyl alcohol hence it fixes and stains the blood at the same time; the time taken is from 10 to 15 minutes. After pouring the stain on the slide and diluting it with water the slide must be kept covered by a bell jar to prevent evaporation of the alcohol.

(ii) Giemsa's method: Original Giemsa's stain is diluted in proportion of 1 drop of Giemsa's to every c.cm. of distilled water. The thin film previous to staining must be fixed with (pure and acetone free) methyl alcohol. It takes about half-an-hour for the blood to be properly stained. The slide may be left in this fluid overnight if necessary.

In the preparation of Giemsa's stain it is necessary to use absolutely neutral medium as the slightest trace of either acid or alkali interferes with the staining reaction. The distilled water must previously be boiled to remove the dissolved CO_2 and cooled before it is used.

In place of distilled water, Giemsa's stain diluted as above can be used for staining slides according to Leishman's method.

(iii) (A) Rapid method of Singh and Bhattacharji (1944).

Composition of the stain.

SOLUTION I

Medicinal methylene blue	0.1 gm.
Potassium dichromate	0.1 gm.
Sulphuric acid, 1.0 per cent by volume	0.6 c.cm.
Potassium hydroxide 1.0 per cent	2.0 c.cm.
Water	100 c.cm.

Dissolve the methylene blue in 100 c.cm. water by constant shaking. Add sulphuric acid and then Potassium dichromate. A heavy purple-coloured precipitate forms. After shaking thoroughly the solution is heated in a water-bath at boiling point. After about 3 hours' heating the colour of the liquid and also of the precipitate becomes greenish and then bluish. At the end of this period the solution is cooled and the precipitate appears as steel-blue needle-like branched crystals. Add 2 c.cm. of 1 per cent. caustic potash solution drop by drop, while shaking the flask continuously; this dissolves the greater portion of the precipitate. The liquid is then filtered several times to ensure complete solution of the dye remaining on the filter paper. The solution can be used after 48 hours.

SOLUTION II

Dissolve 1 gm. of water-soluble eosin in 500 c.cm. of tap water. The preparation should be allowed to mature for about a week.

Method of staining.

(1) Fix the smear in a jar containing methyl alcohol and allow it to dry thoroughly. (2) Dip the slide in solution I for 30 seconds. (3) Dip in acidulated

tap water having pH 6.2 to 6.6 for 2 seconds. (4) Dip in solution II for 1 second. (5) Rinse in the acidulated water for 5 seconds. (6) Dip in solution I for 10 seconds. (7) Again rinse in the acid water for 2 seconds or till the smear shows a pink background. Dry and examine under oil-immersion lens.

(B) Field's method (1941):—Applicable for films neither thin nor thick. The film should not be so thick that objects cannot be almost clearly seen through it. Freshly prepared films after they have properly dried stain best.

SOLUTION I

Methylene blue	0.8 gm.
Azure I	0.5 gm.
Disodium hydrogen phosphate (anhydrous)	5.0 gm.
Potassium dihydrogen phosphate (anhydrous)	6.25 gm.
Distilled water	500 c.cm.

SOLUTION 2

Eosin (yellow eosin, water soluble)	1.0 gm.
Disodium hydrogen phosphate (anhydrous)	5.0 gm.
Potassium dihydrogen phosphate (anhydrous)	6.25 gm.
Distilled water	500 c.cm.

The phosphate salts are first made up in solution in the distilled water and the stains are added. The azure I granules should be mixed with a small quantity of the phosphate solution in a mortar. The solutions are allowed to stand for 24 hours, thereafter they are filtered. In case a scum or a precipitate is noticed, subsequent filtration will be necessary.

There is the possibility of the eosin solution becoming a little greenish. In that case a fresh solution should be used.

Technique :

- (1) Dip film for 1 second in solution 1.
- (2) Rinse immediately in clean water until the stain ceases to flow from the film.
- (3) Dip for 1 second in solution 2.
- (4) Rinse in clear water.
- (5) Place the slide in a vertical position to dry.

Blood films stained by either Singh and Bhattacharji's or Field's method will give as satisfactory results as when other methods are used.

Thick film :

In this method the unfixed thick film is dried for from 2 to 24 hours ; then a mixture of 2 drops of Giemsa's stain to 2 c.c. of water is poured on the slide and allowed to remain for half to one hour. If necessary dehaemoglobinisation may be accelerated by using dilute acetic acid, after which the film is fixed in pure methyl alcohol, stained and washed in water.

A large number of methods for staining thick films have been recommended. Among them Sinton's method (1924) is extensively used in India and gives quite satisfactory results.

Generally both the thick and the thin films are prepared on the same slide, the thick film at one end and the rest of the slide is covered by the thin film.

It is essential that the thin film only should be fixed with methyl alcohol and then allowed to dry. Both thick and thin films are now flooded with Giemsa's stain, diluted with distilled water as stated above, and allowed to remain for half-an-hour. The slide is placed in a vertical position for drying.

Knowles and Das Gupta's method (1924).

It gives excellent results due to the fact that dehaemoglobinisation is very thorough. In place of acid-alcohol for this purpose they recommend acetic and tartaric acids.

The thick film is first treated with a mixture of glacial acetic acid (2.5% soln.)—4 parts and tartaric acid, crystalline (2.0% soln.)—1 part till the blood film becomes dehaemoglobinized. The time by which it is completed depends on the thickness and freshness of the film. The fluid is now drained off and methyl alcohol is poured over the slide but never directly on the film. After the alcohol has been drained off, the film is carefully and thoroughly washed with very slightly alkaline water. The last trace of acid must be removed. It is now stained in the usual way with Giemsa's.

James' modification of Ross's thick film method.

Immerse in a mixture of 10 drops of commercial HCl in 50 c.c. of ethyl alcohol until the haemoglobin is completely dissolved. Then place in tap water for 10 to 20 minutes. Dry in the air. Stain with undiluted Romanowsky for 2 or 3 minutes, then dilute freely, carrying out this dilution in successive stages, the whole process occupying about 10 minutes. Finally wash in tap water until no more blue colour comes from the film.

INFANTILE MALARIA INDEX

The study of the examination of blood for malarial parasites in newly-born infants during the first attack of fever after their birth is useful from several points of view, the most important being the determination of the actual season of transmission of malaria, also whether different seasons are concerned in the transmission of the different species of parasites. By applying this method Strickland and Sen Gupta (1936) came to the conclusion that inoculation of malaria by *A. minimus* in Assam certainly took place in September, October, November and April, and possibly in every month of the year as was later borne out by Rice and Mohan (1936). In the same way Russell, Sweet and Menon (1939) found the transmission season in Southern India to be from July to January, the most active transmission by *A. culicifacies* taking place during the period September to January.

From the malaria parasite indices in infants in Assam, Viswanathan (1941) concluded that *P. vivax* has the transmission period from May to July, and *P. falciparum*, the most prevalent species all through the year in Assam, is especially numerous during June and July.

TRANSMISSION SEASON

The transmission season is determined from (1) the malaria index in newly-born babies, (2) from sporozoite indices in wild mosquitoes correlated with different seasons, and (3) from artificial infectivity results carried out in room conditions.

ARTIFICIAL INFECTION OF ANOPHELES

Mosquitoes may either be bred out from eggs laid in the laboratory or they may be reared from larvae caught in the field. In India where larvae are so easily obtained, the latter method is more convenient. The adult mosquitoes may be sustained on split raisins and cotton wool soaked in water till they are required. Mosquitoes live much longer if kept in a cold place than in the laboratory room, preferably at a temperature of 75°F. in the presence of a moderate amount of humidity.

Whether a volunteer is suitable for infecting mosquitoes will depend principally on the presence of mature gametocytes. Darling (1910) thought that the number was more important and fixed it at 12. It is now held that the presence of the flagellating forms of the parasites will alone determine their development in the stomach of the mosquito provided other conditions are favourable. It is also noticed that gametes may exflagellate *in vitro* and yet fail to infect.

The enumeration of gametocytes is done by Sinton's method (1924) which involves the mixing of a fixed quantity of blood taken in a fine capillary tube with an equal quantity of a suspension of fowl blood cells, the number of blood cells per c.cm. having been previously determined. Thick drops are made from the mixture and stained without fixation. The ratio of the parasites to fowl cells gives the number of gametocytes per c.mm. of blood. The calculation is made as follows:—

$$\frac{A \times B}{C} = \text{number of gametocytes.}$$

A = number of gametocytes counted whilst counting C = the number of fowl cells ; B = the number of fowl cells per c.mm. contained in the emulsion.

The determination of the maturation of the gamete is not difficult. After a blood film, neither thick nor thin, has been rapidly drawn on a slide the latter is placed inside a moist chamber at 25°C. A Petri dish containing a thick pad of blotting paper and well soaked in water will make a good chamber. In order to prevent the blood from drying, it may be necessary to breathe on the slide before it is put in the moist chamber. In the tropics except during the winter season, it will not be necessary to use an incubator. After half-an-hour the slide is withdrawn, fixed and stained with either Leishman or Giemsa. The flagellating gametes can be examined under an oil-immersion lens.

The next step is to introduce either one arm or one leg inside a feeding cage through its sleeve. *Anopheles* mosquitoes are reluctant to feed in the day time. There are exceptions no doubt, e.g., *A. stephensi*, which bite at all hours of the day. The raisin and the water should be withdrawn in the day time and the mosquitoes are fed at night in the dark. Those which have taken blood feed are caught in test-tubes and kept separately in another cage, cotton wool soaked in

water and split raisin being provided. From time to time dissections are performed with a view to determining if they have become infected.

A feeding cage is not convenient to use in the case of children or very sensitive patients. For them, the mosquitoes may be enclosed inside a small lamp chimney, both ends of which are closed by a piece of mosquito netting tied round the edge. The wider end is applied against the skin of the back or the abdomen at the time of feeding.

It is necessary to point out that if other conditions are favourable, one blood meal will be sufficient to infect mosquitoes. For artificial infectivity experiments we never allow the mosquitoes more than one feed on a volunteer.

The following factors are known to influence the infectivity of a mosquito:—

(1) The unknown intrinsic factors which favour the complete development of the parasites in certain mosquitoes, partial development in some, and absence of development in others.

(2) *Density of gametocytes.* Different observers have noted different numbers of gametocytes as the minimum necessary to infect mosquitoes with different species of *Plasmodia*. Thus Strickland, Roy and Chaudhuri (1933), and Knowles and Basu (1943) give 40 per c.mm. in the case of *A. stephensi*. Green (1929) succeeded in infecting *A. maculatus* in Malaya with only 10.

(3) The simultaneous presence of male and female gametocytes in equal numbers in the peripheral blood has been stressed by Boyd (1930).

(4) The flagellating forms of gametocytes may be taken as an indication of their maturity and the presence of mature gametes is perhaps more important than immature forms.

(5) The coexistence of suitable temperature and humidity is regarded as the principal dominating factor influencing the infection in the mosquito. The findings recorded by Strickland, Roy and Chaudhuri (1933) clearly indicate that mosquitoes cannot be infected during April, May and June in Calcutta as the temperature is too high. Although Bentley (1911) had laid stress on humidity more than on temperature as a factor in the infection of mosquitoes, later observers like Gill (1921) and Wenyon (1926) regarded temperature as a much more important factor than humidity, provided the latter is not too low to endanger the life of the mosquito seriously.

(6) In order that the parasites may develop to the sporozoite stage the mosquito must live for at least 10 to 12 days.

INFECTIVE DENSITY OF ANOPHELINES

Davey and Gordon (1933) have described a method for use in interpreting the anopheline density and sporozoite infection rate of infective anophelines. Infective density is the average number of infective anophelines per room per night.

Samples should be taken from different houses in the early morning and in order to calculate the number of females that were present in the houses during the night, it may be necessary to increase the figure by adding an estimated amount (expressed in the formula by "X") representing the number that had left the houses before examination. The anopheline density is therefore the number of female anophelines captured + "X" divided by the number of rooms examined. The infec-

tive ratio is the number of females with sporozoites divided by the number dissected. The infective density is then obtained by multiplying the anopheline density by the infective ratio. If "X" is known, the infective densities of localities in different parts of the world can be compared.

These workers point out that whereas the individual risk of inoculation with malaria cannot be estimated accurately from the anopheline infective density, yet the former depends on the latter. A close correlation has been found to exist between a high infective density and a high malaria rate amongst children examined during the first three months of their life, while a low infective density is associated with a low infective rate.

THE PRECIPITIN TEST

The precipitin test is the only means of obtaining exact information of the feeding habits of blood-sucking insects. It is a valuable method of studying insect transmission of disease, particularly of malaria. This method of determining the origin of unknown blood in insects was introduced by Uhlenhuth and his collaborators (1908).

The principle of this test is that when clear serum extracted from dried blood from the stomach of insects is brought into contact with a series of sera containing precipitins for blood of the possible animal sources, a precipitation or flocculation occurs in the specimen in which the blood and antiserum are homologous. At present the ring test of Fornet and Müller (1910) is universally applied for the detection of the precipitation.

The production of precipitating sera.

Selection of animal—Though rabbits are universally employed for this purpose, fowls have also been used in this country. The quantity of serum recovered from a fowl is too small and a high mortality among the experimented birds is generally experienced. In Calcutta Belgian rabbits have shown better response than the ordinary rabbit and for tropical conditions these rabbits should be preferred.

The selected animal is injected with the blood serum of the animal for which precipitins are required.

Dosage of the serum—Although a single injection of a large quantity may yield more reliable results with the serum of a large number of animals, for the successful production of anti-human serum repeated injections are necessary and for this purpose a minute quantity for each injection will bring about the desired results. The total quantity of serum necessary to immunize a rabbit is seldom more than 4.2 c.cm. and sometimes less.

The injections should be given in the marginal ear vein of the rabbit and the dosage employed in fractional quantities is 0.1 c.cm. daily during the first week, 0.2 c.cm. during the second week, and 0.3 c.cm. during the third week. During the third week the blood should be examined frequently to gauge the degree of precipitating action developed by the animal, as further injections of the antigen should be discontinued as soon as the maximum titre is reached. If injections are continued without interruption, it may lead to the total disappearance of the precipitins or it may lead to the production of strong heterologous antibodies in addition to homologous ones.

If the strength of the serum does not conform to the standard (see later), a further course of injections of 0.4 c.cm. daily may be given during the fourth week.

During the period of immunization the animals should be given plenty of carrots; lettuce and cabbage should be withheld as the latter are thought to be responsible for the turbidity of the serum.

The work of precipitin production should always be started during the cold weather when a very large percentage of animals will generally give a satisfactory response; during summer the response is extremely poor.

Collection of blood—The animal should be starved for about 18 hours before the blood is collected, and an hour before the blood is withdrawn it should be allowed to drink plenty of water, the object being to obtain not only clear but also a large quantity of the serum.

The practice usually followed in drawing out the blood from an immunised animal is to open the carotid artery and to bleed it to death.

Instead of collecting all the blood at one time, we advocate the withdrawal of 20 c.cm. of heart blood at an interval of 4 to 5 days as long as the potency of the serum has not sufficiently deteriorated to warrant its rejection. Accidental deaths from heart puncture are not very common, though they do at times occur. In this way it will be possible to recover a much larger quantity of serum than would have been possible if a single collection had been made by opening the carotid artery.

Further if it is proved that the immunity response is dependent on the mechanism of the antibody producing organs, which according to our findings is inherent in the animal, it therefore pays to use a satisfactory animal more than once.

From 9 to 12 days after the last injection about 50 c.cm. of blood is collected from the same rabbit by cardiac puncture. Two days later a second bleeding of the same amount may be made. After a rest of about a week, the whole process is repeated. In many instances rabbits are given as many as five series of injections and bled twice following each series. Anaphylactic reactions are avoided by giving a preliminary injection of 1 c.cm. of the serum subcutaneously the day preceding the beginning of the second and subsequent series of injections. In this way one can obtain more than 200 c.cm. of potent precipitating serum from each rabbit.

As far as possible the syringe should be dry and this can be effected by washing with ether. For separating the serum from the clot, test-tubes are preferable to flasks.

The serum should be preserved in 1 c.cm. sealed ampoules in cold storage.

The precipitins are fairly stable bodies and if stored in a refrigerator will keep for a long time.

The question whether or not a preservative should be added to the serum before it is stored in sealed tubes, can be answered by stating that the development of contamination does not lower markedly the titre of the serum.

When blood from an immunized animal is collected in a tube, the serum that separates out within the first 24 hours is generally a clear fluid. If further attempts are made to collect the rest of the serum that separates on the second and third days, it is likely to be blood tinged on account of haemolysis. The reaction obtained with coloured serum is also clearly defined as with clear serum. Red blood cells should be completely excluded by centrifugalization from any serum, be it tinged

or clear, as their presence will cause a varying amount of turbidity when such anti-serum comes in contact with the antigen.

Testing precipitating serum—For the purpose of testing the serum the ring test of Fornet and Müller (1910) is employed. The antigen is first introduced by means of a capillary pipette into a small glass tube having a diameter of 3 to 4 mm., and thereafter the precipitating serum is cautiously run along the wall of the tube by means of a separate pipette. A 'ring' forms at the site of contact between the antigen and the serum.

The potency of the precipitating serum is judged merely by the reaction time, *i.e.*, the earliest time within which a well defined ring appears. Its character is judged by testing with both homologous and heterologous antigens. The standard to discriminate useful from useless serum is considered from the following:—

Precipitating serum + Homologous antigen diluted 1: 1,000 = Definite ring within 2 to 3 minutes.

Precipitating serum + Heterologous antigen diluted 1: 500 = No reaction within 5 minutes.

We advocate the above as a standard representing the character of a useful serum which should be strictly followed in the determination of the feeding habits of insects.

A precipitating serum may be either specific or non-specific. These terms are, however, relative because a serum will often yield a quick specific and a delayed non-specific reaction. When both specific and non-specific reactions appear with only a short interval between them, the serum is as a rule useless for practical purposes unless it is found that when the precipitating serum has been diluted with salt solution, the interval between the two reactions is considerably increased, but the diluted serum must still yield the characteristic reaction with the homologous antigen within 2 to 3 minutes.

In order to economise material the bulk of the precipitating serum can be suitably increased by dilution with normal salt solution taking care that this does not in any way reduce its potency below the standard laid down above. It is, however, emphasised that for practical tests of mosquito blood or blood ingested by other insects, the quantity of which is unknown, the precipitating serum should never be diluted to the maximum limit with a view to minimising the chances of missed reaction; therefore fairly strong though diluted serum should be preferred. It is further emphasized that the serum should always be preserved as such, and that any dilution necessary for economy should be done just before use.

Stomach meal preparations.

Method of preparation—For this purpose only those insects should be chosen which have recently fed on blood and in which its presence in the stomach can be detected from outside. As soon as the specimen has been identified and killed, the blood meal should be pressed out by crushing the abdomen on a piece of filter paper with a solid glass or metal rod. The expressed blood is soon absorbed by the filter paper. All traces of blood should be removed from the glass rod by wiping before the next specimen is dealt with. Each 'blood spot' on the filter paper should bear the same number as the insect. The filter papers are stored in

a small covered jar, preferably in a dark cool place, and when a large number have been collected, they are to be tested for the determination of the sources of their blood meals.

Blood feeding insects have proteolytic enzymes of the tryptic type in their midgut. The longer the process of digestion is allowed to continue, the more the blood will be acted upon by the enzyme. The chances of detecting the origin of blood in their alimentary canal thus become correspondingly minimized.

Specimens in which digestion has advanced considerably should be examined as soon after preparation as possible.

The process of digestion is considerably delayed in cold surroundings, and for this reason the transportation of living mosquitoes, if they are required for precipitation tests, from the field to the laboratory must be made in tubes kept inside a thermos flask containing ice.

THE PERFORMANCE OF THE TEST.

The principle involved in practical tests is to bring the antigen and the precipitating serum in actual contact without allowing the two to mix with each other. Positive reactions are indicated by the appearance of a white ring within the specified time at the site of contact. As dual reactions with precipitating sera and three reactions with anti-human serum (man, dog and cattle) are common, the time limit must be strictly enforced in order to avoid overlapping of any of these reactions. The standardization of the serum extracted from the dried blood is impossible and it is for this reason that we advocate the use of fairly strong precipitating serum though it may be diluted.

The two methods which are commonly applied for testing unknown blood of insects are separate examination in serological tubes and examination *en masse*.

The first is well known and was extensively used till Rice and Barber (1934) advanced an improved method which allows examination of a large number of specimens within a short space of time. A brief resume of Rice and Barber's technique is given below. The essential apparatus required for this test are the following:—

(1) A metal tray divided into a number of compartments, each compartment measuring 1 cm. \times 3 cm., being 2 mm. wide at the bottom and considerably wider at the top; these are used for soaking the dried blood preparations separately in salt solution.

(2) A mixture containing sodium chloride (4.25 gm.), glycerine (166 cc.), phenol (2.5 cc.) and distilled water (330 cc.) for diluting the precipitating serum according to its titre.

(3) A number of capillary tubes, each about 6.5 cm. long and 2 mm. inside diameter. Five such tubes, sealed between two glass slides, are termed a 'card.'

(4) Five small pots for holding the diluted antisera prepared against five different types of animals.

(5) The procedure adopted is to dip the tubes placed on the 'card' in one compartment of the tray in which the extract of the dried blood in salt solution has been prepared.

All the five tubes are quickly filled up to variable lengths with the same type of antigen. The ends of the tubes are then touched to a layer of wet absorbent cotton-wool to draw out a part of the antigen. The tubes are next dipped into the dishes containing five different types of precipitating sera, which at once rise into the tubes. The pots containing the antisera are placed in a line at such a distance from each other that, when the card is lowered on the pots, the five tubes will draw in fluid separately from the five pots; thus the different tubes will contain the five different types of antisera.

The zonal reaction at the contact between the antigen and the precipitating serum should be read up to 20 minutes.

This method of testing unknown blood *en masse* no doubt presents certain definite advantages. It economizes time and also precipitating serum. It cannot, however, be considered as perfect and certain deficiencies which are experienced in its practical application may be pointed out.

Stomach meal preparations of mosquitoes prepared by crushing the insect on filter paper often cover quite a large area, and these spots may often have to be cut up into smaller bits in order to accommodate them in a tray having a width of 2 mm. at the bottom, especially when only a few drops of salt solution are required to extract serum from the dried blood. In the tropics the loss of fluid due to evaporation from trays, unless they are kept covered, is great. The cleaning of capillary tubes and their washing while still fixed to the glass card cannot be conveniently performed. In order to allow the capillary action to continue after the tubes have been loaded with the antigen, which is the extracted serum in this instance, a small amount of the contents must be expelled which, as has been advised by Rice and Barber, should be done by touching the ends of the tubes with wet cotton-wool. The fluid is so quickly drawn out from tubes having 2 mm. inside diameter, that one must be extremely careful to prevent their complete emptying. In actual practice such accidents have often occurred in our hands, necessitating a repetition of the whole process. Though the authors advise mere contact of the ends of the tubes with the fluids, both the antigens and the precipitating sera, in practice one really dips the ends of the tubes in the fluid which necessarily adheres to the side. The danger of contamination of the precipitating serum cannot be eliminated by merely touching the end of the tube with wet cotton-wool.

The above technique has been modified by Roy and Ganguli (1943). They claim that they have been able to overcome the drawbacks and to simplify the application of the test still further. Details of their technique are given below:

1. Dilution of the precipitating serum.

In order to minimize the expenditure of the serum, the highest dilution in which it reacts with the homologous antigen within the specified period must first be determined so that the bulk of the serum can be proportionately increased by diluting with salt solution. Weak sera must be used in the concentrated form. The estimated amount that is likely to be consumed in the course of a day should be prepared.

It is essential that the serum must be tested immediately before it is actually put into use.

2. Only the actual blood spots are to be cut out from the filter paper. These are now allowed to soak in normal saline solution for two hours in separate test-tubes in a rack, the latter being shaken from time to time. The solutions are then poured into separate hollow glass blocks of small size just before use. The glass blocks are arranged in a regular order so that their labelling can be avoided.

3. Antisera, either concentrated or diluted, are poured also into separate glass blocks, and these should be kept covered.

4. Testing tubes are specially prepared from Pasteur's capillary pipettes. A. large portion of the upper part and much of the capillary part, except the first $1\frac{1}{2}$ to 2 inches, are cut off, and the sharp edges are rounded to avoid accident.

5. These tubes are left in ordinary tap water or distilled water, if available, and before they are used, the water is expelled by jerking. Even a minute column of water, especially in the narrow end, will interfere with the capillary action.

6. The actual test now begins. Immediately after the water has been expelled from the tube, the capillary end is first dipped in the mosquito blood solution, when the fluid at once rushes up to a distance depending on the internal diameter of the tube. This end is now quickly drawn through a piece of towel held in the left hand to wipe off the fluid from the outside of the tube. It is now touched to a piece of filter paper and nearly half of the contents are allowed to run out. The capillary end is next dipped in the precipitating serum which is at once drawn inside. The tube is now put into a specially prepared wooden rack with suitable holes to accommodate the ends of the tubes and with sufficient height that the capillary end of the tube when put in the rack does not touch the base. In this way 50 tubes are loaded; this will take not more than 5 minutes. Their reading should not be postponed later than 5 minutes, and this can best be done by holding the rack and examining the tubes against a black card board. If the room is not well lighted, suitable arrangements for artificial light should be made. Only positive reactions are entered in the book. Occasionally a closer scrutiny is necessary in which case the particular tube will have to be taken out. When the test does not give any conclusive result, the particular specimen may have to be re-examined.

So far only one antiserum has been used, and the examinations have to be

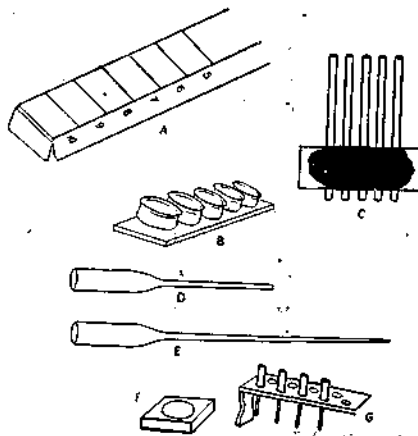


Fig. 43

Apparatus for precipitation test. A, B, and C after Barber and Rice and D, E, F and G after Roy and Ganguli. A, Tray for making saline emulsion of dried blood meal. B, Pots containing different anti-precipitating sera. C, A card of testing tubes. D, Testing tubes prepared from Pasteur's pipette. E, F, Glass block. G, Testing tubes placed on a rack.

repeated with other antisera. In this way the examination of 50 samples at a time are performed with the different antisera.

It is necessary to mention that in handling the loaded tubes a little shaking does not disturb their contents.

If the capillary end of the tube is wide, the lower meniscus of fluid may project outside. This can be easily obviated by using pipettes which have fine capillary ends about 1 mm. in diameter. Too fine capillary tubes should never be used, as it is difficult properly to recognize the white ring.

In order to assess the possible advantages of the above method one has to judge it from the viewpoint of its practical application. If a large number of antisera have to be used, Rice and Barber's method has definite advantages, but when the number of animals against which the tests have to be performed is small the new technique outlined above is particularly convenient and easy to apply.

In malarial entomology in India it should be emphasized that the use of only two sera, *e.g.*, anti-cattle and anti-human will generally serve all practical purposes.

In order to maintain uniformity it is suggested that the preferential feeding habits should be indicated not from the total number subjected to precipitin tests but from those which give positive reactions against different animals, a practice now followed by the large majority of workers.

IDENTIFICATION OF FEMALE ANOPHELES SPECIES OF INDIA

GENERAL CHARACTERS OF A MOSQUITO.

(a) It is a delicately built midge-like insect. (b) It has long filamentous many jointed antennæ. (c) Proboscis is long and adapted for piercing the skin and for sucking blood. (d) Body and wings are clothed in scales. (e) Wing venation is characteristic, e.g., the second, fourth and fifth longitudinal veins are branched. (f) Presence of a row of scales (wing fringe) along the whole length of the posterior border of the wing.

IDENTIFICATION OF FEMALE ANOPHELINE MOSQUITOES.

Sexes are differentiated from antennæ which are plumose in males and pilose in females.

The female *Anopheles* has long, rod-like palpi whereas in the male *Anopheles* the palpi are clubbed at the end.

INSTRUCTIONS FOR EXAMINATION OF ANOPHELINE MOSQUITOES FOR IDENTIFICATION.

(a) It is very convenient to examine a mosquito when it has been mounted on a pin.

(b) When there are no facilities for pinning, it can be examined on some teased cotton wool. The legs and wings are very important for identification and therefore they must be fully stretched taking care that this does not cause any damage to either the leg or the wing. It must be remembered that scales on the wings when rubbed off will render identification extremely difficult.

The specimens are examined with a hand lens X 15 unless a dissecting microscope is handy. Examination of minute structures like hairs and scales is always made under $\frac{2}{3}$ objective of a compound microscope.

(c) It is essential that mosquitoes should always be examined in subdued light. When examined in ordinary light banding and speckling may be missed. It may even be necessary to interpose the hand or a paste board in order to cut off light.

(d) For the identification of an unknown *Anopheles* proceed first with the key and for confirmation use the table.

(e) Mosquitoes have been placed under six different groups according to certain distinctive characters common to every member of that group.

(f) Certain terms must be properly understood before any attempt is made in the examination of a mosquito.

(i) Banding on palpi. There are generally three pale bands on each palp and these are arranged as follows. When examined from the tip of the palp there are two white bands placed near to each other and close to the apex; the third band is placed near the base. The first band is known as the apical pale band and the second band the subapical pale band. (Fig. 33). In a few species there are four pale bands on the palp.

(ii) Speckling of legs and palpi. Speckling is due to numerous minute white spots on a dark background.

(iii) Base of wing is that part of the wing which is in contact with the thorax. The basal part of the costa means that part of the anterior border of the wing which lies in close proximity to the thorax.

(iv) White-footed mosquitoes, *i.e.*, those which have the last tarsal segment of the hind legs white.

(v) Unless otherwise mentioned abdominal scales mean scales on the dorsum of the abdomen.

(vi) "Subcostal pale area" is the pale area situated almost at the middle of the anterior border of the wing. This is of some importance in the identification of *A. annularis*.

(vii) Hair. It has a round circumference and is of nearly uniform thickness throughout.

Scale. A scale is a modified hair and is flat.

N.B.—All figures relating to the identification of *Anopheles* adults refer to those on pp. 97, 98, and 99.

TABLE FOR THE IDENTIFICATION OF ADULT *ANOPHELES* SPECIES OF THE FEMALE SEX ONLY OF INDIA

Mosquitoes have been placed under six different groups according to certain distinctive characters common to every member of that group.

GROUP I.

Chief characters.—Costa uniformly dark, *i.e.*, not interrupted by any pale spot; also no white spot on the wing field.

A. Conspicuous white scaling on vertex of head; milk-white frontal tuft present; white scales on the anterior third of the mesonotum; distinct white banding at the distal end of hind femur. *A. bariensis*. (Fig. 1.)

B. No white scaling on vertex of head; white frontal tuft absent; mesonotum of uniform colour and scaleless; banding at the distal end of hind femur absent.

(a) Head scales very long, narrow, erect and notched at the free end; anterior forked cell much larger than the posterior; long filamentous palpi.

A. aitheni. (Fig. 2.)

(b) Head scales normal and broad; anterior forked cell slightly larger than the posterior.

A. culiciformis.

GROUP II.

Chief characters.—Less than 4 dark spots involving the costa, subcosta and the first longitudinal vein.

A. Palpi with distinct pale bandings.

(a) Inner quarter of costa with pale interruptions ; a large pale spot on the wing fringe between veins 5.2 and 6. *A. gigas*. (Fig. 3.)

(b) Inner quarter of costa dark without any pale interruptions ; wing fringe between veins 5.2 and 6 dark ; a broad golden spot at the tip (apex) of the wing ; the proximal half of vein 6 pale. *A. hyrcanus* var. *nigerrimus*. (Figs. 4 & 35).

(In *A. hyrcanus* var. *sinensis* a fringe spot at 5.2 present.)

(c) Presence of prominent tuft of scales, black above and white below, about the femoro-tibial joint of hind legs. *A. annandalei*. (Fig. 25).

B. Palpi unbanded.

(a) Hind femur with a conspicuous broad white band.

A. lindesayi. (Figs. 5 & 26).

(b) Hind femur without any band.

(i) Palpi shaggy ; fringe spot at 5.2 ; scattered black scales on the proximal half of vein 6 ; presence of both dark and white scales on wing veins ; 7th abdominal segment with a conspicuous tuft of black scales on the ventral surface.

A. barbirostris. (Figs. 6, 19, & 36).

(ii) Palpi thinner ; fringe spot at 5.2 absent ; proximal half of vein 6 pale ; only dark scales on the wing field ; 7th abdominal segment without tuft of scales.

A. umbrosus.

GROUP III.

Chief characters.—At least 4 dark spots involving the costa, subcosta and the first longitudinal vein.

A. Apex of palpi dark (not definitely white).

(a) Only 2 indefinite dark spots on vein 6, the distal one being very long ; extreme base of costa dark ; complete absence of dark scales on the mesonotum.

A. turkhudi. (Figs. 8 & 37).

(b) Three definite dark spots on vein 6 ; extreme base of costa pale ; scattered scales on mesonotum.

A. multicolor. (Fig. 7).

(c) Palpi inconspicuously banded ; mesonotum scaleless ; no pale scales on wing veins except on costa and vein 1 and no fringe spot present. Head scales narrow and erect.

A. dthali.

B. Apex of palpi white.

I. Two broad pale bands of equal length at the apex, the intervening dark area being narrow.

(a) Costa with one pale interruption at the base ; no fringe spot at vein 6 ; proboscis uniformly dark.

A. minimus. (Figs. 9 & 38).

(b) The basal area of the costa usually with an interruption ; fringe spot at vein 6 present ; proboscis golden at its distal half. *A. aconitus*. (Figs. 11 & 40).

(c) The basal area of the costa without any interruption ; no fringe spot at vein 6 ; proboscis may have a light golden appearance at the distal part.

A. varuna. (Figs. 10 & 39).

II. The pale apical band nearly of the same size or a little broader than the subapical pale band, the intervening dark area being much broader than either of the two bands.

(a) Nearly the whole of the third vein dark.

(i) Only two fringe spots 4.2 and 5.1 present ; basal dark area on the costa interrupted by a white spot opposite which the first longitudinal vein is dark ; mesonotum scaleless.

A. culicifacies. (Figs. 12 & 41).

(ii) Fringe spots at all veins except the 6th ; basal dark area on the costa uninterruptedly dark opposite which there is a large pale area on the first longitudinal vein ; palpi comparatively long, about $1\frac{1}{2}$ times the length of the thorax.

A. sergenti.

(b) Nearly the whole of 3rd vein pale.

(i) Inner third of costa uninterruptedly dark. No fringe spot opposite vein 6.

(a) 2 dark spots on vein 6, the distal one being fairly long ; tibio-tarsal joints dark ; scales confined to the anterior third of the mesonotum.

A. fluviatilis. (Figs. 13 & 42).

(β) 3 dark spots on vein 6 ; tarsal joints of front legs narrowly banded ; white scales distributed over the whole mesonotum along the middle line.

A. moghulensis. (Fig. 43).

(ii) Inner third of costa interrupted by pale spots.

(a) Tarsal joints of front and midlegs narrowly banded white ; white scales in the form of a tuft in the middle of the mesonotum ; fringe spot at vein 6 ; 3 dark spots on vein 6.

*A. jeyporiensis** (Figs. 14, 32 & 44).

(β) Tarsal banding and mesonotal tuft absent ; no fringe spot at vein 6 ; 2 dark spots on vein 6, the distal one being fairly long ; palpi long and thin ; mesonotum heavily scaled.

A. superpictus. (Fig. 47).

III. The pale apical band very much broader than the subapical pale band, the intervening dark area being either of the same size as the apical pale band or smaller than the latter ; front tarsal joints broadly banded. A prominent T-shaped dark spot involving the costa, subcosta, and the first vein about the middle of the anterior border of the wing.

(a) The dark intervening area of the same length as the pale apical area.

A. subpictus. (Figs. 15, 27 & 46).

(b) The dark intervening area extremely narrow, even less than half the apical pale area.

A. vagus. (Fig. 48).

**A. jeyporiensis* var. *candidiensis* is distinguished from the type form by markings on the palp. (Fig. 45).

GROUP IV.

Chief characters.—At least 4 dark spots on the costa, etc., as in Group III.

Femora and tibiae speckled.

A. Three pale bands on the palp. 3 or less than 3 dark spots on vein 6.

(a) Two equally broad white bands at the apex, the intervening dark area being very small; palps speckled (easily seen in fresh specimens); thoracic scales very broad. *A. stephensi*. (Fig. 49).

(b) The apical pale area is much broader than the subapical pale band which is very narrow, and the former is of almost the same length as the intervening dark area. Thorax is mostly hairy no broad scales being present. *A. sundaicus*. (Fig. 50).

B. Four pale bands on the palp. More than 3 dark spots on vein 6.

(a) Broad tibio-tarsal white band on the hind leg. One broad and three narrow, white bands on the palp as seen from the tip to the base. *A. leucosphyrus*. (Figs. 24 & 51).

(b) Three broad apical and one narrow band; the three apical bands being placed close to each other and separated by two narrow black bands; apical half of proboscis golden yellow. *A. tessellatus*. (Fig. 52).

GROUP V.

Chief characters.—At least 4 dark spots on the costa etc. as in previous groups.

Femora and tibiae not speckled.

A. At least the third, fourth and fifth tarsal segments of hind legs completely white.

(a) 5th vein mainly dark with a dark spot at its bifurcation, the dark area extending over the anterior branch for a short distance; the subcostal pale area bridged by a dark area on vein 1; a conspicuous white spot at the end of segment 1 of hind tarsus. *A. annularis*. (Figs. 16 & 29).

(b) 5th vein extensively pale without the dark spot on the stem at its bifurcation; the subcostal pale area not bridged by a dark area on vein 1.

(i) Distal end of segment 1 of hind tarsus inconspicuously marked white; no white scales on ventral aspect of abdomen except on last 2 or 3 segments only; scaling on the dorsum of the abdomen confined to the last two or three segments. *A. philippinensis*. (Figs. 17 & 30).

(ii) No white spot at the end of segment 1 of hind tarsus; broad white scales scattered over ventral aspect of most of the abdominal segments; scaling on the dorsum of abdomen extends over the last 5 or 6 segments. *A. pallidus*. (Figs. 18 & 31).

(c) Conspicuous white scales on the dorsum of the thorax and abdomen, the scale tufts projecting laterally from each abdominal segment.

A. pulcherrimus. (Figs. 34 & 55).

B. Only tarsus 5 and one-third of tarsus 4 of hind legs completely white.

(a) Three pale bands on the palp. The two apical bands are broad and equal. *A. majidi*. (Fig. 53).

(b) Four pale bands on the palp. The three distal bands are broad and the proximal one is very narrow. *A. karwari*. (Figs. 28 & 54).

GROUP VI.

Chief characters.—At least 4 dark spots on the costa etc.

Femora and tibiae speckled.

A. At least the third, fourth and fifth tarsal segments of hind legs completely white.

(a) The apical pale band broad, the subapical pale area being narrow ; palps not speckled.

(i) At least the last two segments of the abdomen covered with golden scales ; wings pale ; a yellowish mosquito. *A. jamesi*. (Fig. 56).

(ii) No golden scales on the abdomen ; wings much darker ; a small dark mosquito. *A. ramsayi*.

(b) Two equally broad pale apical bands ; palpi speckled.

A. splendidus. (Fig. 57).

(c) Four pale bands on the palp ; conspicuous white scales on the dorsum of the abdomen and the thorax ; lateral scale tufts projecting from each abdominal segment. *A. pulcherrimus*.

(*A. pulcherrimus* may have speckled front legs).

B. The whole of fifth and fourth tarsal segments of hind legs completely white ; no dark band on fourth tarsal segment. *A. theobaldi*. (Fig. 22).

C. Fifth and a third of fourth tarsal segments of hind legs completely white ; a dark band on the fourth tarsal segment present.

(a) Abdominal scales only on the last 2 or 3 segments.

A. maculatus. (Figs. 23 & 58).

D. Only half of the last tarsal segment of hind legs white ; four pale bands on the palp ; prominent tuft of black scales on the ventral surface of each abdominal segment ; outer half of proboscis golden. *A. kochi*. (Figs. 20 & 21).

Key to the identification of Anopheles (females only) of India.

Group I.

Costa uniformly dark, i.e., not interrupted by any pale spot ;
also no white spot on the wing field.

Anterior forked cell much larger than
the posterior.

A. aitheni.

Anterior forked cell of nearly the same
size as the posterior.

Distinct white
banding at the
distal end of hind femur.
Frontal white scale
present.

A. bariensis.

Banding absent.

A. culiciformis.

Scale tuft

Group II.

Less than four dark spots involving the costa, subcosta and vein 1.

Palpi with distinct pale bandings.		Palpi unbanded.	
A prominent tuft of scales, black above and white below, about the femoro-tibial joint of hind legs.	No such tuft of scales present.	Hind femur with a conspicuous broad white band.	Hind femur without any white band.
	<i>A. annandalei</i> .	<i>A. lindesayi</i> .	
Inner quarter of costa with marked pale interruptions. Wing fringe between 5½ and 6 white. A brightly coloured mosquito.	Inner quarter of costa dark. Tip of wing golden. A dark mosquito.	Palpi shaggy. Fringe spot at 5½ present. Presence of both dark and white scales on wing veins. A common species.	
	<i>A. hyrcanus</i> var. <i>nigerrimus</i> .	<i>A. barbirostris</i> .	<i>A. umbrosus</i> .
<i>A. gigas</i> .			Fringe spot at 5½ absent. Only dark scales on the wing field. A rare species in India.

Groups III—VI have at least four dark spots involving the costa, subcosta and vein I.

Group III.

Dark-footed series (of hind legs only). Femora and tibiae not speckled.

Tips of palpi dark.

Wing veins except on costa and V.1 contain only dark and white scales.

A. dthali.

2 indefinite dark spots on V6, the distal one being very long. *A. multicolor*.

A. turkhuhi.

Tips of palpi pale.

V3 mostly dark.

Only 2 fringe spots 4.2 and 5.1 present. (Palpi of normal length).

A. culicifacies. *A. sergenti*.

V3 mostly pale

The two apical pale bands are of equal or nearly equal lengths and the intervening dark area is small.

The two pale apical bands are definitely unequal.

No fringe spot at V6.

Basal area of the costa dark without any pale interruption.

A. varuna.

A. minimus.

Fringe spot at V6.

A. aconitus.

The intervening dark area on the palp is much larger than either of them.

The intervening dark area is either of the same size as the apical pale band or very much smaller.

Inner third of costa un-interruptedly dark.

Inner third of costa interrupted.

The dark area of the same size as the apical pale area.

A. subpictus. *A. vagus*.

2 dark spots on V6. Tibio-tarsal joints dark.

A. fluviatilis.

3 dark spots on V6. Tibio-tarsal and tarsal joints of front legs narrowly banded.

A. moghulensis.

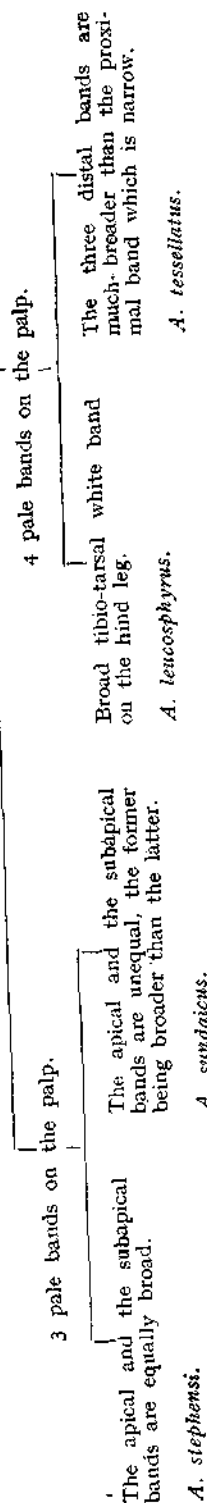
Tarsal joints of leg I banded. Fringe spot at V6.

A. jeyporiensis. *A. superpictus*.

Tarsal banding of leg I absent. No fringe spot on V6.

Group IV.

Dark-footed series (hind legs only). Femora and tibiae speckled.



Group V.

White-footed series (hind legs only). Femora and tibiae not speckled.

Only tarsus 5 and 1rd. of tarsus 4 of hind legs completely white.

At least tarsal segments 3, 4 and 5 of hind legs completely white.

3 pale bands on the palp.

A. majidi.

4 pale bands on the palp.

A. karwari.

Vein 5 mainly dark with a dark spot at its bifurcation.

A. annularis.

Vein 5 extensively pale and no dark spot at the bifurcation.

Distal end of tarsus 1 of hind legs inconspicuously marked white.

A. philippinensis.

Distal end of tarsus 1 of hind legs dark.

A. pallidus.

Conspicuous white scales on the dorsum of the abdomen and thorax.

A. pulcherrimus.

Group VI.

White-footed series (hind legs only). Femora and tibiae speckled.

<p>1 of tarsus 5 of hind legs white. Prominent scale tufts on the ventral surface of each abdominal segment.</p> <p><i>A. kochi</i>.</p>	<p>Whole of tarsus 5 and one-third of 4 of hind legs white with a dark band on tarsus 4.</p> <p><i>A. maculatus</i>. <i>A. willmori</i>.</p>	
	<p>Whole of tarsi 5 and 4 of hind legs completely white.</p> <p><i>A. theobaldi</i>.</p>	
	<p>2 equally broad pale apical bands; p a l p i speckled.</p> <p><i>A. splendidus</i>.</p>	<p>The two pale apical bands unequal.</p>
	<p>At least the last two segments of the dorsum of the abdomen covered with golden scales.</p> <p><i>A. jamesi</i>.</p>	<p>Dark scales on the abdomen.</p> <p><i>A. ransayi</i>.</p> <p>Conspicuous white scales on the dorsum of the thorax and abdomen.</p> <p><i>A. pulcherrimus</i>.</p>

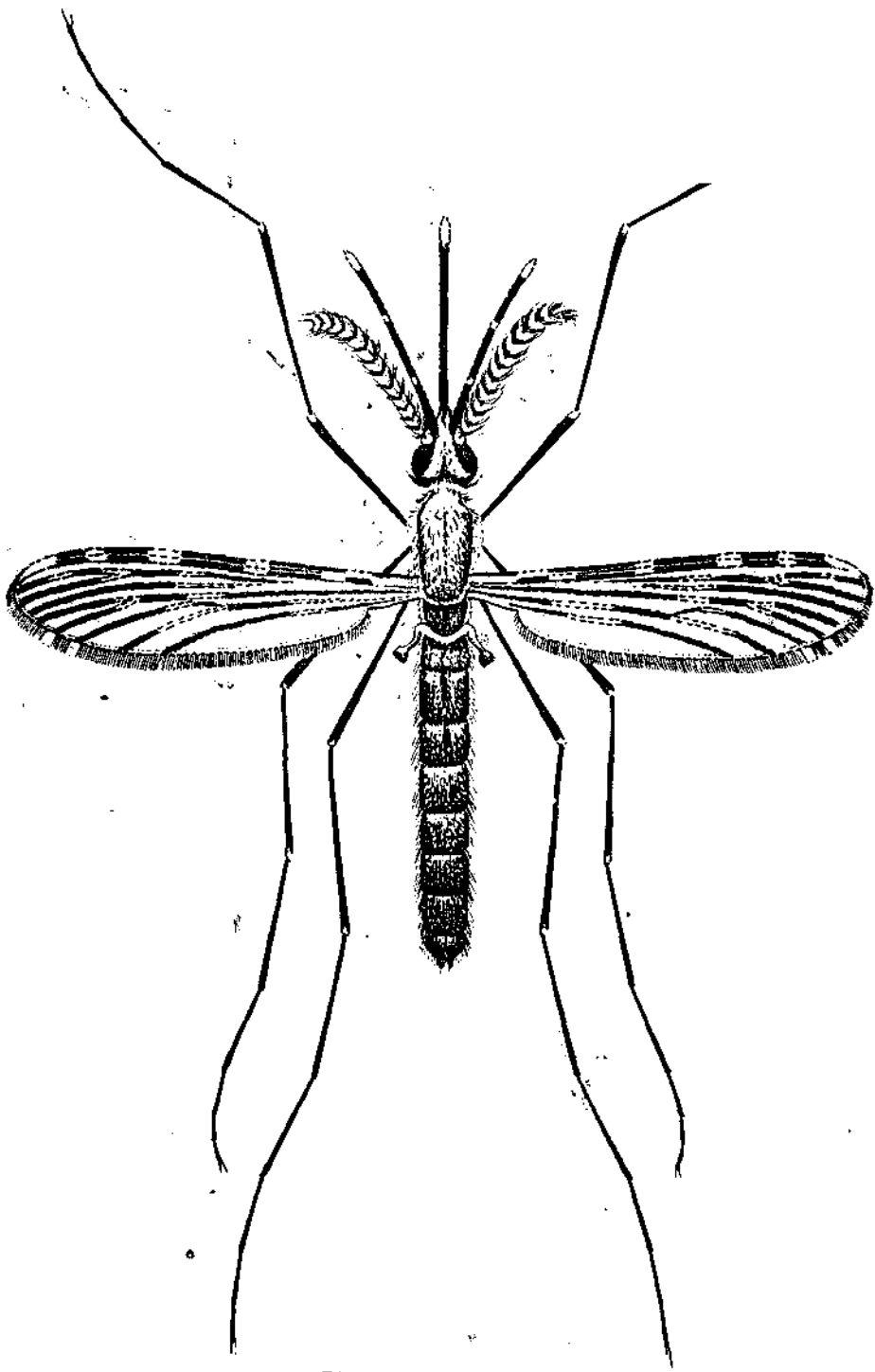


PLATE I

Anopheles culicifacies.

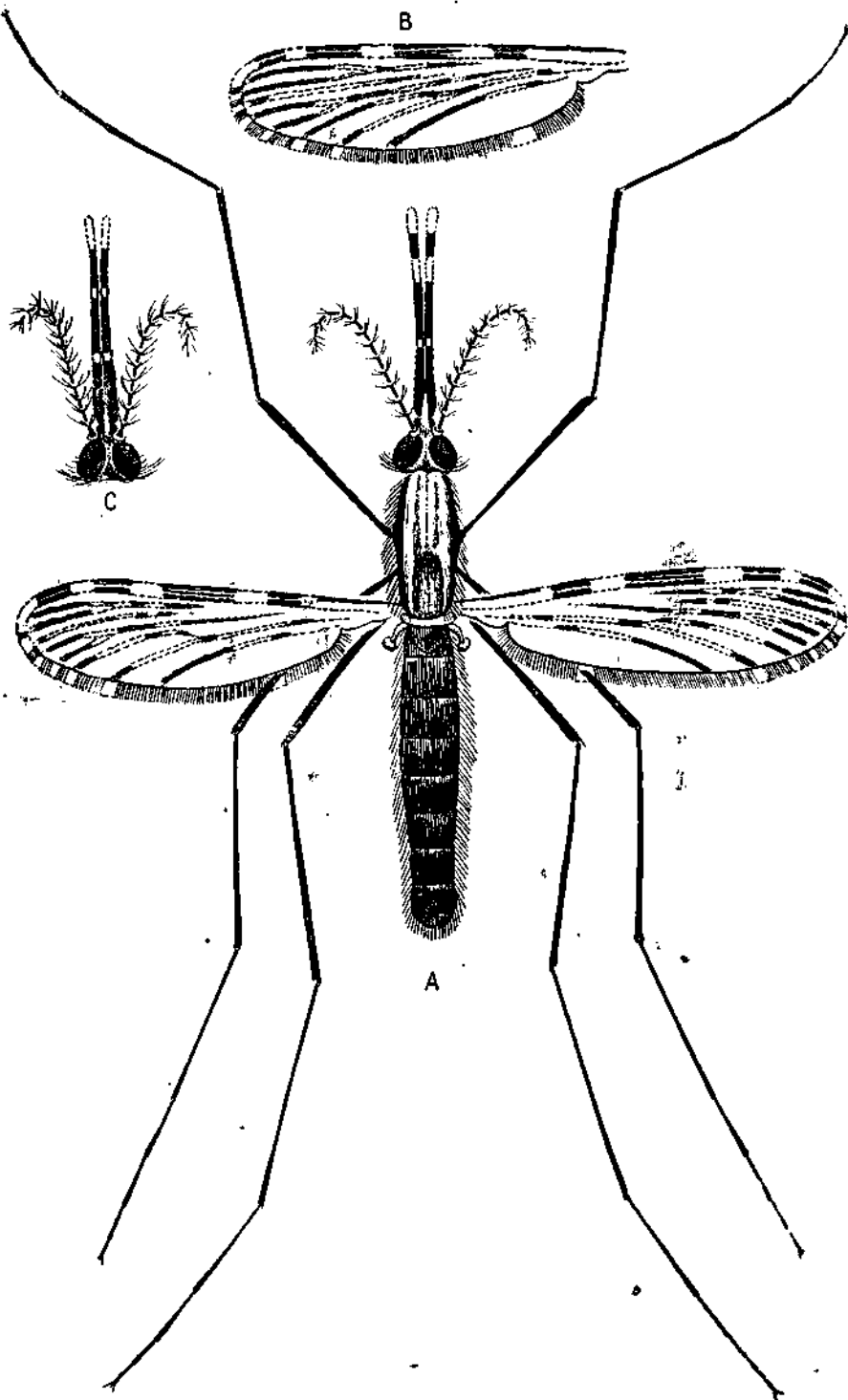


PLATE II.

A. *Anopheles minimus*.
B. & C. Wing and head of *A. fluviatilis*.

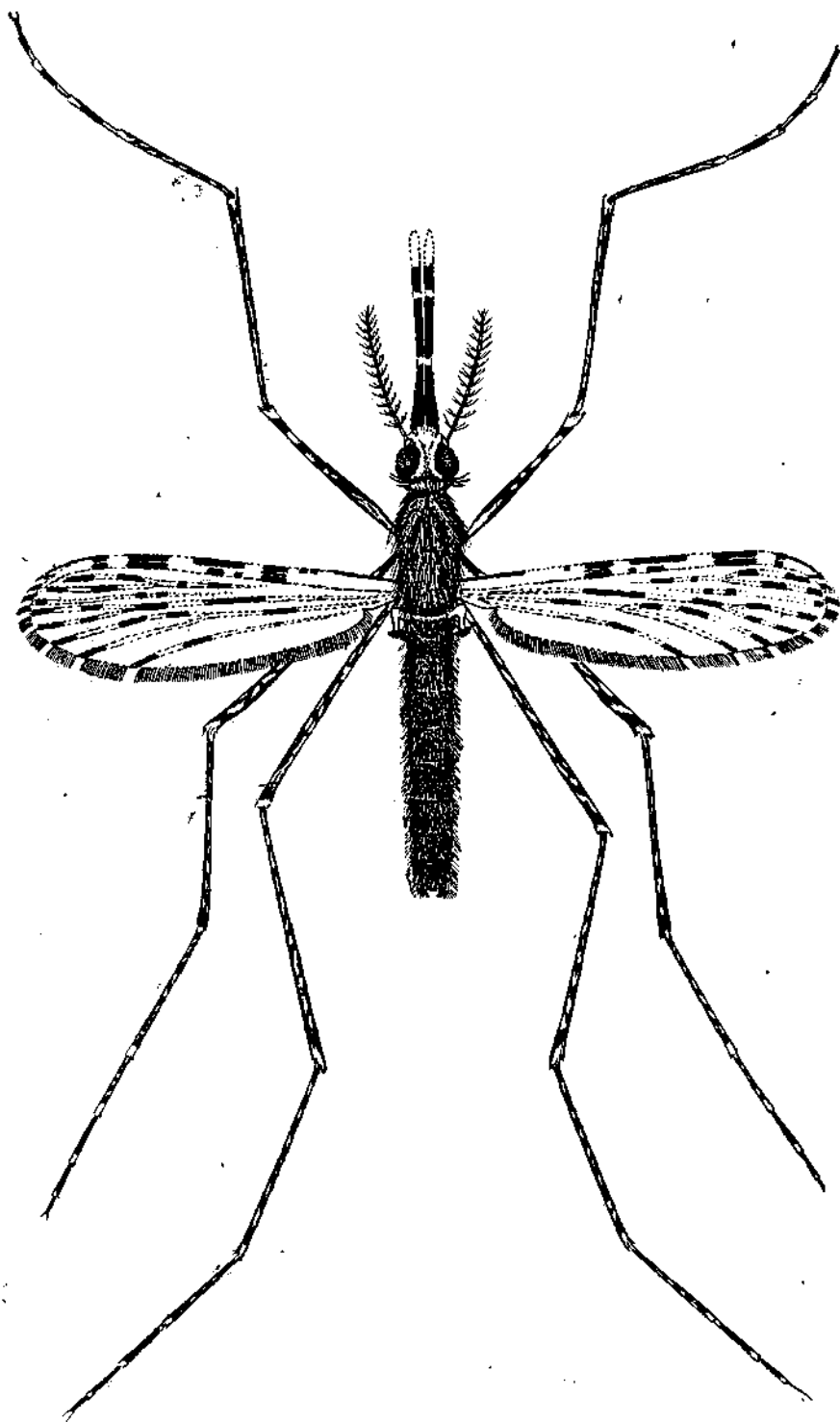


PLATE III.
Anopheles sundanicus.

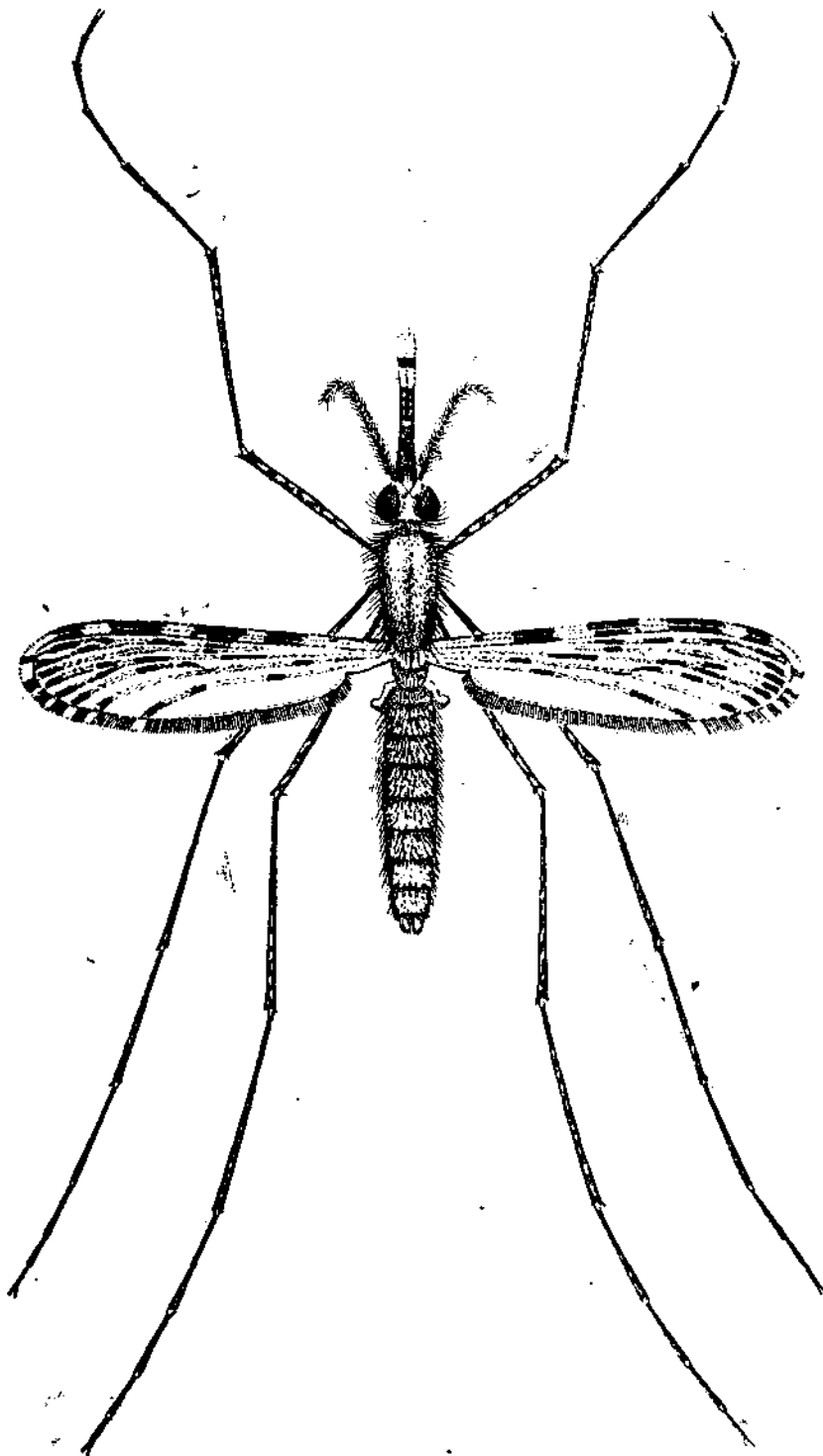


PLATE IV.

Anopheles stephensi?

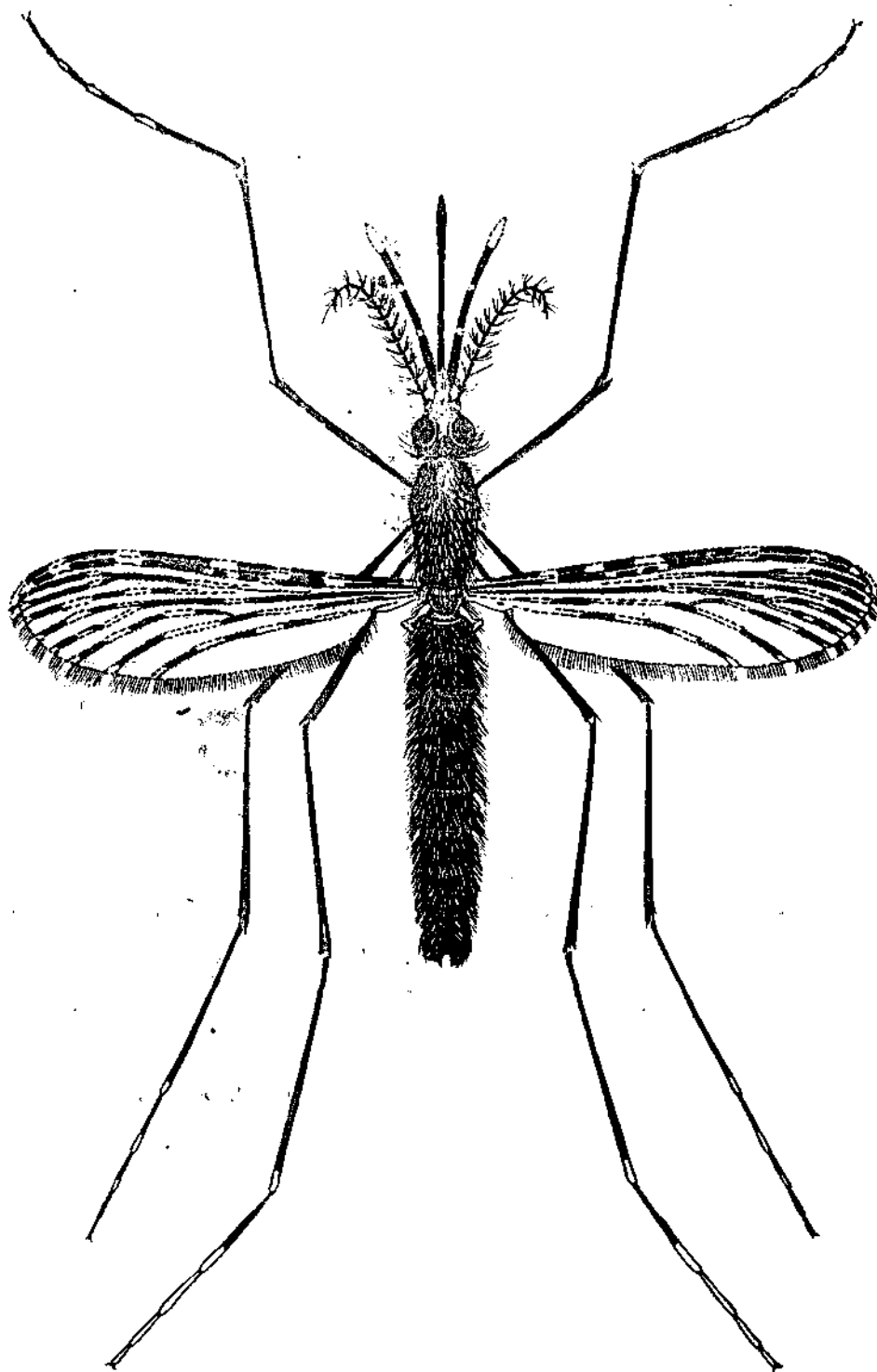


PLATE V.

Anopheles annularis.

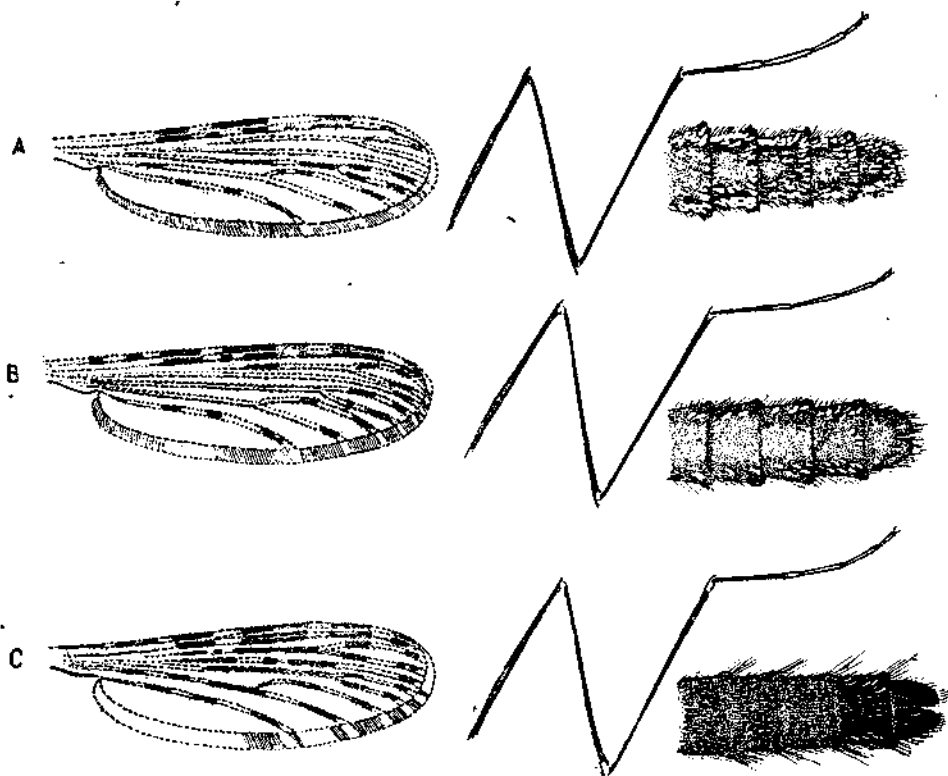


PLATE VI.

Wing, hind leg and dorsum of abdomen of (A) *A. pallidus*,
(B) *A. philippinensis* and (C) *A. annularis*.

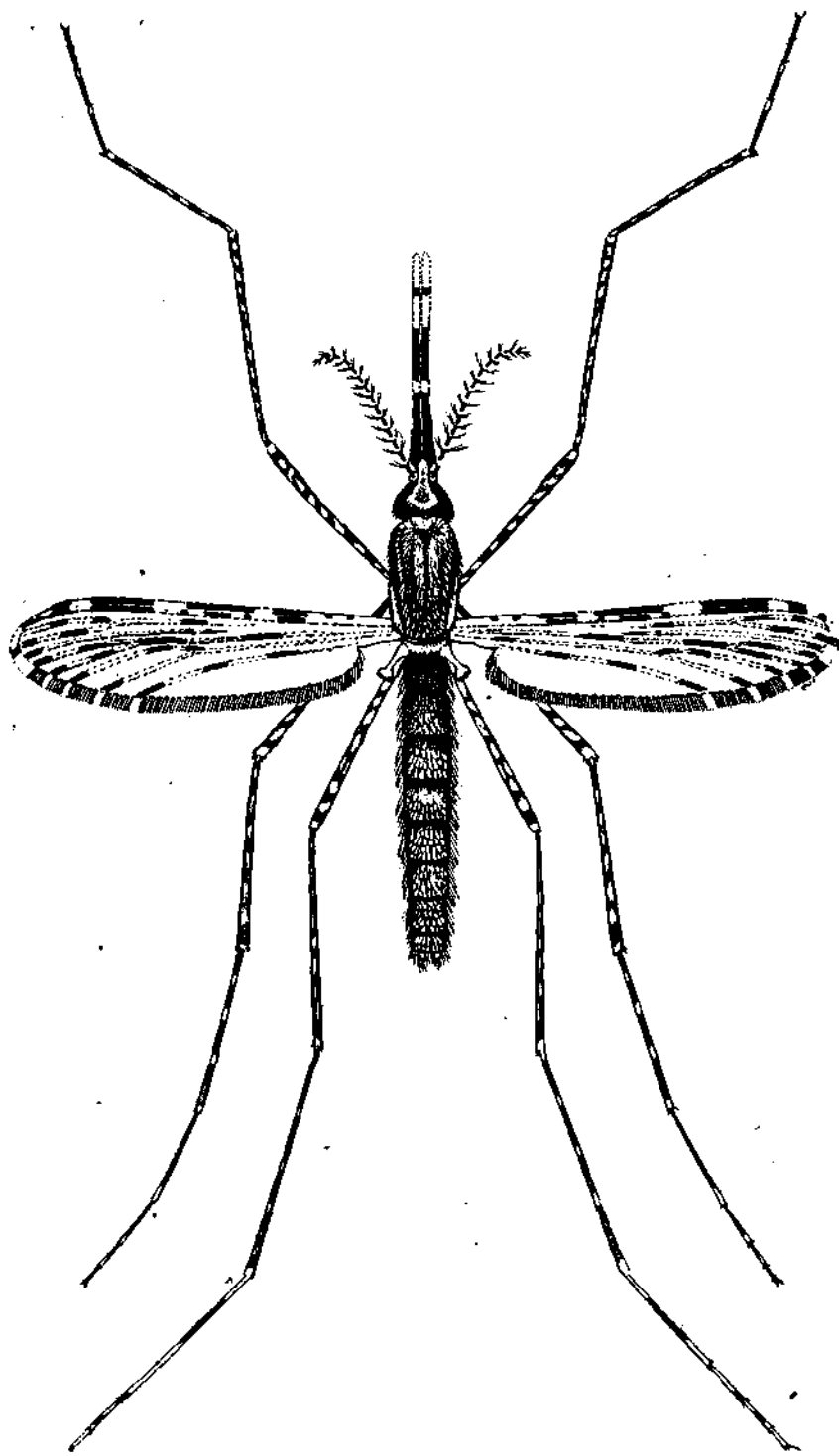


PLATE VII.

Anopheles maculatus.

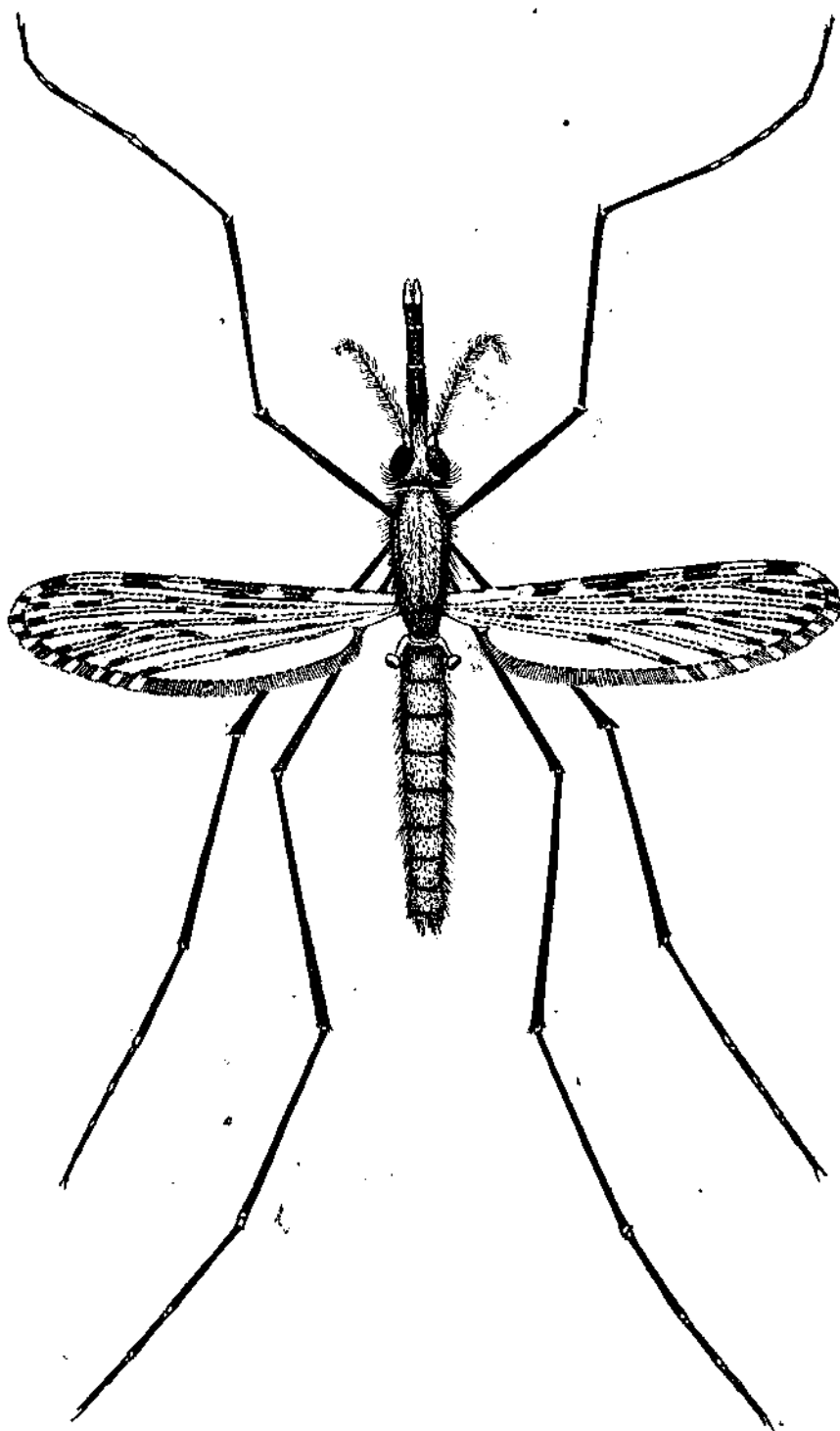


PLATE VIII.
Anopheles subpictus.

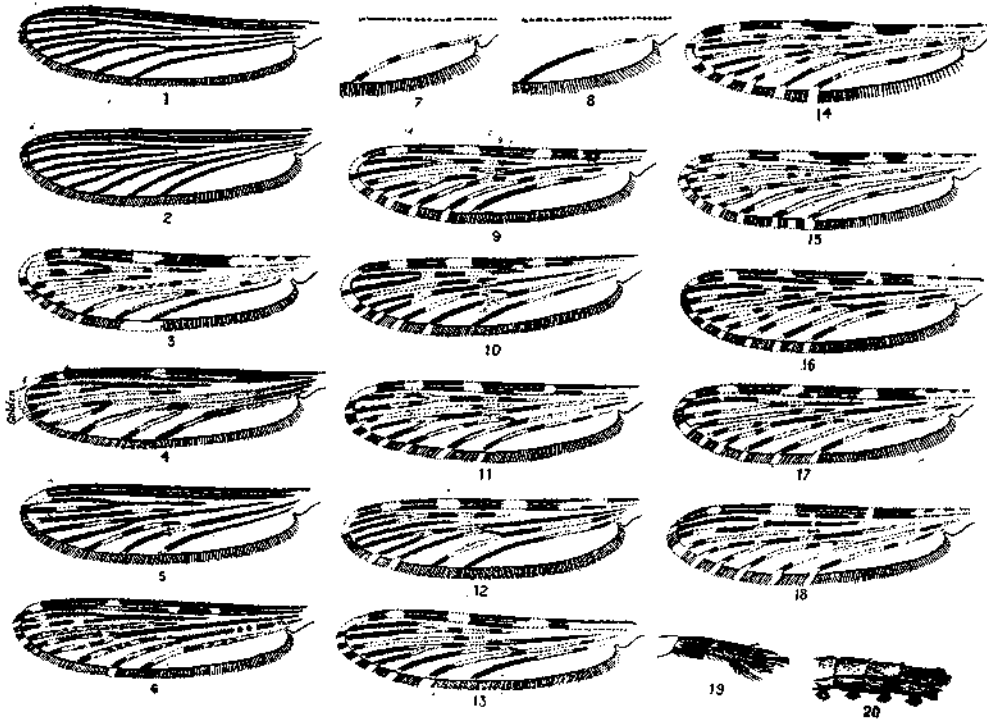


Fig. 44

- | | |
|--|---|
| 1. Wing of <i>A. barianensis</i> . | 11. Wing of <i>A. aconit-rs</i> . |
| 2. „ „ <i>A. aitkeni</i> . | 12. „ „ <i>A. calceifacies</i> . |
| 3. „ „ <i>A. gigas</i> . | 13. „ „ <i>A. fluviatilis</i> . |
| 4. „ „ <i>A. hyrcanus</i> var. <i>nigerrimus</i> . | 14. „ „ <i>A. jeyporiensis</i> . |
| 5. „ „ <i>A. lindesayi</i> . | 15. „ „ <i>A. subpictus</i> . |
| 6. „ „ <i>A. barbirostris</i> . | 16. „ „ <i>A. annularis</i> . |
| 7. „ „ <i>A. multicolor</i> . | 17. „ „ <i>A. philippinensis</i> . |
| 8. „ „ <i>A. turkhudi</i> . | 18. „ „ <i>A. pallidus</i> . |
| 9. „ „ <i>A. minimus</i> . | 19. Abdomen of <i>A. barbirostris</i> . |
| 10. „ „ <i>A. varuna</i> . | 20. „ „ <i>A. kochi</i> . |

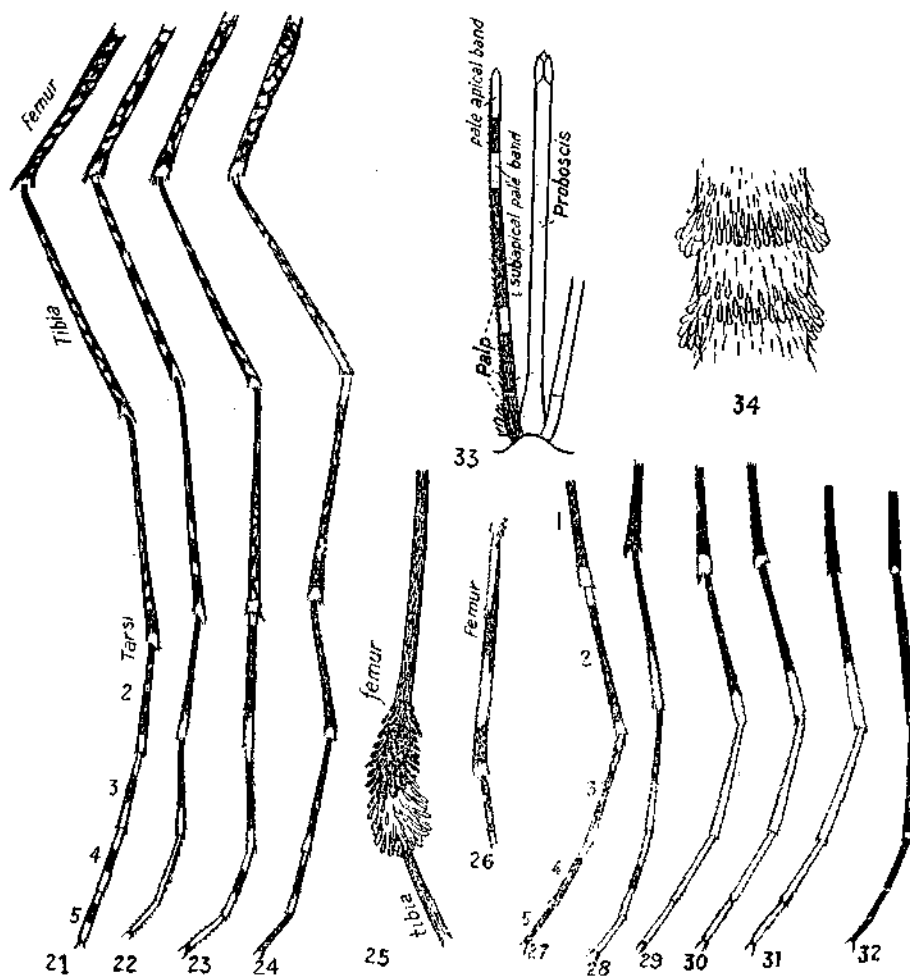


Fig. 45

- | | |
|--|---|
| 21. Hind leg of <i>A. kochi</i> . | 28. Hind foot of <i>A. karwari</i> . |
| 22. " " " <i>A. theobaldi</i> . | 29. " " " <i>A. annularis</i> . |
| 23. " " " <i>A. maculatus</i> . | 30. " " " <i>A. philippinensis</i> . |
| 24. " " " <i>A. leucosphyrus</i> . | 31. " " " <i>A. pallidus</i> . |
| 25. " " " <i>A. annandalei</i> . | 32. Foreleg of <i>A. jeyporiensis</i> . |
| 26. Femur of <i>A. lindesayi</i> . | 33. Proboscis and palp of mosquito. |
| 27. Frontal tarsal joints of <i>A. subpictus</i> . | 34. Abdomen of <i>A. pulcherrimus</i> . |

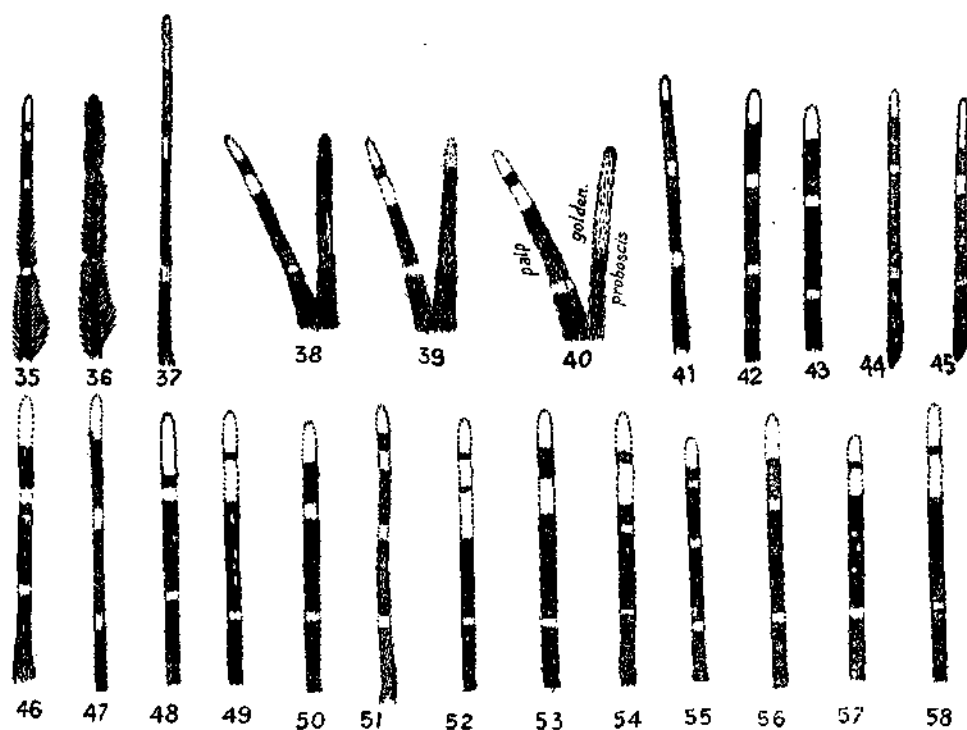


Fig. 46

- | | |
|---|----------------------------------|
| 35. Palp of <i>A. hyrcanus</i> var. <i>nigerrimus</i> . | 47. " " <i>A. superpictus</i> . |
| 36. Palp of <i>A. barbirostris</i> . | 48. " " <i>A. vagus</i> . |
| 37. " " <i>A. turkhudi</i> . | 49. " " <i>A. stephensi</i> . |
| 38. Palp and proboscis of <i>A. minimus</i> . | 50. " " <i>A. sundaicus</i> . |
| 39. " " " " <i>A. varuna</i> . | 51. " " <i>A. leucosphyrus</i> . |
| 40. " " " " <i>A. aconitus</i> . | 52. " " <i>A. tessellatus</i> . |
| 41. Palp of <i>A. culcifacies</i> . | 53. " " <i>A. majidi</i> . |
| 42. " " <i>A. fluvialis</i> . | 54. " " <i>A. karwari</i> . |
| 43. " " <i>A. moghulensis</i> . | 55. " " <i>A. pulcherrimus</i> . |
| 44. " " <i>A. jeyporiensis</i> , type. | 56. " " <i>A. jamesi</i> . |
| 45. " " <i>A. jeyporiensis</i> var. <i>candidiensis</i> . | 57. " " <i>A. splendidus</i> . |
| 46. " " <i>A. subpictus</i> . | 58. " " <i>A. maculatus</i> . |

IDENTIFICATION OF ANOPHELINE LARVÆ.

METHODS OF EXAMINATION OF ANOPHELINE LARVÆ

1. They may be examined in the living state in a drop of clean water and covered by a cover glass.

2. Dead specimens are mounted in a drop of carbolic acid and covered by a cover glass. In this way a large number of specimens can be examined within a short time.

3. The specimens may be permanently mounted on a glass slide. It is absolutely useless for the purpose of identification if the mosquito larva has not been mounted with the dorsal surface pointing upwards. Clypeal hairs, palmate hairs, etc. lie on the dorsal surface and are always examined with $\frac{1}{8}$ objective.

CHARACTERS OF THE DORSAL AND VENTRAL SURFACES OF ANOPHELINE LARVA

Examination is made in a good drop of either carbolic acid or lactophenol under $\frac{2}{3}$ rd. objective of a compound microscope. The specimen may or may not be covered by a coverglass. On the dorsal surface lie the clypeal hairs, frontal hairs, antennae, palmate hairs and spiracles, whereas on the ventral surface are the mandibles, mentum and the maxillary palps. Therefore in order to determine whether the dorsal or the ventral surface lies uppermost, the object is placed under the microscope and focussed and if the mouth organs and the maxillary palps are seen first, the larva has to be turned with a needle in order that the dorsal surface may point upwards.

N.B.—All figures relating to the identification of Anopheline larvae refer to those on pp. 114 and 115.

HOW TO PROCEED WITH THE IDENTIFICATION

(1) Find out under which group the mosquito larva can be placed. To facilitate identification anopheline larvæ have been placed under seven separate groups on the basis of certain distinctive features.

(2) Then consult the key.

(3) The table should be referred to whenever necessary or when confirmation of the identification already made with the help of the key is required.

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Table for the identification of fourth stage or mature Anopheline larvae of India.

(A few have been left out as they are not only uncommon but are also unimportant).

GROUP I.	GROUP II.	GROUP III.	GROUP IV.	GROUP V.	GROUP VI.	GROUP VII.
<p>(a) Internal clypeal hairs placed close together.</p> <p>(b) Antennal hair branched and placed dorso-externally.</p> <p>(c) Frontal hairs long and thickly branched. (Figs. 1 & 45.)</p> <p><i>A. aitheni</i> type. <i>A. aitheni</i> var. <i>A. gigas</i>. <i>A. hindesayi</i>. <i>A. barbirostris</i>. <i>A. hyrcanus</i> var. <i>nigerimus</i>. <i>A. umbrosus</i>.</p>	<p>(a) Internal hairs placed close to each other.</p> <p>(b) Antennal hair simple and placed dorso-externally.</p> <p>(c) Most of the frontal hairs are short and simple or sparingly branched. (Figs. 18 & 46.)</p> <p><i>A. annandalei</i>. <i>A. bariensis</i>. <i>A. culiciformis</i>. <i>A. sintoni</i>.</p>	<p>Anterior abdominal tergal plates exceptionally well developed. (Figs. 7 & 8.)</p> <p><i>A. aconitus</i>. <i>A. jeyporiensis</i>. <i>A. minimus</i>. <i>A. fluvialis</i>. <i>A. varuna</i>.</p>	<p>(a) Internal clypeal hairs so finely and sparingly frayed that they appear simple.</p> <p>(b) The external hair is nearly one-quarter the length of the internal.</p> <p>(c) The posterior clypeal hair placed far posteriorly.</p> <p>(d) Shape of mid-abdominal tergal plates characteristic.</p> <p>(e) The filamentous part of the mid-abdominal palmar hairs is hazy and has a ground glass-like appearance. (Figs. 25, 26 & 30.)</p> <p><i>A. kochi</i>. <i>A. tessellatus</i>. <i>A. leucosphyrus</i>.</p>	<p>Internal clypeal hairs finely but conspicuously frayed.</p> <p><i>A. maculatus</i>. <i>A. kargari</i>. <i>A. stephensi</i>. <i>A. moghulensis</i>. <i>A. superpictus</i>. <i>A. pulcherrimus</i>. <i>A. theobaldi</i>.</p>	<p>Internal clypeal hairs thickly frayed.</p> <p><i>A. annularis</i>. <i>A. philippinensis</i>. <i>A. pallidus</i>. <i>A. janesi</i>. <i>A. ramisayi</i>. <i>A. splendidus</i>.</p>	<p>Internal clypeal hairs simple.</p> <p><i>A. subpictus</i>. <i>A. culicifacies</i>. <i>A. vagus</i>. <i>A. majidi</i>. <i>A. sundanicus</i>. <i>A. stephensi</i>. <i>A. multicolor</i>. <i>A. turktradi</i>. <i>A. dthah</i>. <i>A. sergenti</i>.</p>

GROUP I.

- Chief characters:*
- (a) Internal clypeal hairs placed close together.
 - (b) Antennal hair branched and placed dorso-internally
 - (c) Frontal hairs long and thickly branched.
- (Figs. 1 & 45).

1. *A. aitkeni*.

A jungle species and found mainly in Assam.

The two common forms are (1) *A. aitkeni* type and (2) *A. aitkeni* var. *bengalensis*.

A. aitkeni type.

- (a) Internal clypeal hair bifid.
- (b) External hair short and branched.
- (c) Posterior hair a short tuft the branches arising close to the root.

A. aitkeni var. *bengalensis*.

- (a) Internal clypeal hair has more than 2 branches.
- (b) External hair longer than in the type form and has also more than two branches. (Fig. 1).

2. *A. gigas*.

A hill species found at high altitudes.

- (a) Internal clypeal hair simple.
- (b) External hair simple but occasionally may have one or two fine branches.
- (c) Posterior hair very small, either simple or bifid.
- (d) Abdominal palmate hair developed on segments 3-7, the filament being poorly differentiated.
- (e) Metathoracic hair not differentiated. (Fig. 2).

3. *A. lindesayi*.

Recorded from places at high altitudes.

- (a) Internal and external clypeal hairs long and simple.
 - (b) Posterior hair short and simple or may be bifid.
 - (c) Abdominal palmate hairs developed on segments 2-7, the filament being long and sharply pointed.
 - (d) Metathoracic palmate hair well developed.
- (Fig. 3).

4. *A. barbirostris*.

Widely distributed in India.

- (a) Internal clypeal hair long and simple.
 - (b) External hair characteristically branched.
 - (c) Posterior hair small and branched.
 - (d) Inner submedian prothoracic hair branched from the root.
- (Figs. 4 & 10).

5. *A. hyrcanus* var. *nigerrimus*.

Widely distributed in India.

- (a) Clypeal hairs same as in *barbirostris*.
 - (b) Inner submedian prothoracic hair simple; occasionally the tip may be bifid or trifid.
- (Figs. 4 & 11).

6. *A. umbrosus*. (a) Internal clypeal hair long and simple.
 A very rare species recorded from dense forests in Assam. (b) External hair branched more or less dichotomously with about 7-10 long branches.
 (c) Posterior hair a short tuft with generally three branches arising from the base.
 (d) Abdominal palmate hairs undeveloped on any segment. (Fig. 5).

GROUP II.

- Chief characters.* (a) Internal clypeal hairs placed close to each other.
 (b) Antennal hair simple and placed dorso-externally. (Fig. 46).
 (c) Most of the frontal hairs are short and simple or sparingly branched. (Fig. 18).

1. *A. annandalei*. (a) Internal clypeal hairs long and simple.
 Recorded from Darjeeling forest. (b) External hairs branched.
 (c) Inner frontal hairs simple and very long.
 (d) Innumerable minute spines on the thorax and abdomen even visible under the low power.

2. *A. barianensis*. (a) All clypeal hairs simple.
 An Himalayan species occurring at high altitudes. (b) Frontal hairs larger with one or two branches. (Fig. 18).

3. *A. culiciformis*. (a) Clypeal hairs like those of *barianensis*.
 Confined to Southern India. (b) Frontal hairs larger with one or two branches.

4. *A. sintoni*. (a) Clypeal hairs like those of *culiciformis*.
 Southern India. (b) Frontal hairs have 5-7 branches.

N.B.—All are tree-hole breeders.

GROUP III.

- Chief characters.* Anterior abdominal tergal plates exceptionally well developed. (Figs. 7, 8 & 13).

1. *A. aconitus*. (a) Both internal and external clypeal hairs stout and branched, the branches giving the appearance of thorns.
 Confined to the eastern and southern areas of India. (b) Posterior hair with generally three branches arising from the root. (Figs. 6 & 8).

2. *A. jeyporiensis*. (a) Both internal and external clypeal hairs thickly branched.
Distributed in the eastern and southern areas of the Peninsula. (b) Posterior hair has two or three branches a short distance from the root.
(c) Abdominal tergal plate has a different appearance from that of *aconitus*, *minimus* or *varuna*. (Its posterior border is concave and it does not include the small posterior tergal plate). (Figs. 7 & 9).
3. *A. minimus*. (a) Internal and external clypeal hairs stout, simple and long.
Extremely common in Eastern India, e.g., Assam and Dooars; found also in South India. (b) Posterior hair long and simple.
N.B.—See below under *A. varuna* for point of distinction between *minimus* and *varuna*. (Figs. 8 & 12).
4. *A. fluviatilis*. Difficult to distinguish from *A. minimus*.
A foot-hill species and widely distributed in India.
5. *A. varuna*. Resembles *A. minimus* even in minute details but the presence of a minute branched hair on the tergal plate one on each side of the middle line is characteristic of this species.
In *minimus* this hair is placed near the postero-lateral angle of the tergal plate.
(The internal clypeal hair is so finely frayed in this species that it often appears simple). (Fig. 13).
- GROUP IV.
- Chief characters.* (a) Internal clypeal hairs so finely and sparingly frayed that they appear simple.
(b) The external hair is nearly one-quarter the length of the internal.
(c) The posterior clypeal hair placed far posteriorly.
(d) Shape of mid-abdominal tergal plates characteristic.
(e) The filamentous part of the mid-abdominal palmate hairs is hazy and has a ground glass-like appearance. (Figs. 25, 26 & 30).
1. *A. kochi*. Inner submedian prothoracic hair without any prominent root and with five to ten branches.
A jungle species found in Assam. (Figs. 25 & 26).

2. *A. tessellatus*. Inner submedian prothoracic hair with less than four branches, the root being quite conspicuous. (Figs. 27 & 28).
Recorded from eastern and southern India.
3. *A. leucosphyrus*. Inner submedian prothoracic hair with a prominent dark root and more than ten branches. (Fig. 29).
A wild mosquito recorded mainly from Assam and South India, also from Ceylon.

GROUP V.

- Chief characters.* Internal clypeal hairs finely but conspicuously frayed. (Fig. 31).
1. *A. maculatus*. (a) Both internal and external hairs finely frayed.
Recorded from the (b) Posterior long and simple and is often slightly bent Himalayan areas, hilly towards one side which is very characteristic.
parts in South India (c) Metathoracic palmate hair undeveloped.
also from Ceylon and (d) Abdominal palmate hairs developed on segments 3-7, the filament being long. (Figs. 31 & 32).
breeds in hill streams.
2. *A. karwari*. Closely resembles *A. maculatus* but the filaments are practically absent and blunt at the tip. (Figs. 33 & 34).
Assam and Southern India.
3. *A. stephensi*. See under group VII.
(It may occasionally show minute frayings.)
4. *A. moghulensis*. (a) Internal clypeal hairs stout and very minutely frayed.
Found in hill streams in (b) Both external and posterior hairs long and simple.
North Western and Cen- (c) Metathoracic palmate hair well developed.
tral parts of India. (d) Abdominal palmate hairs well developed on segments 2-7, the filament being long with pointed ends. (Fig. 24).
5. *A. superpictus*. (a) Internal clypeal hair slender and minutely frayed.
Baluchistan and N.W.F. (b) External and posterior hairs long and simple.
Province. (c) Metathoracic palmate hair well developed.
(d) Abdominal palmate hairs as in *moghulensis*.
6. *A. pulcherrimus*. (a) Internal clypeal hairs long and finely frayed.
North-Western India. (b) External hair has a few long fine branches arising from its distal extremity.
(c) Posterior hair short and has 2-3 branches from a short stalk.
(d) Metathoracic hair well developed.

7. *A. theobaldi*.
Scattered especially over
Southern India.

Similar to *A. maculatus*.

8. *A. willmori*.
Confined to the eastern
Himalayas.

Also similar to *A. maculatus*.

GROUP VI.

Chief characters.

Internal clypeal hairs thickly frayed. (Fig. 14).

1. *A. annularis*.
Widely distributed in
India.

- (a) Internal clypeal hair stout, long and thickly frayed.
- (b) External clypeal hair long and characteristically branched.
- (c) Posterior hair with two to three branches at the end of a short stalk.
- (d) Internal sutural hair long and simple or bifid at the tip.
- (e) Palmate hair on the first abdominal segment well differentiated. (Figs. 14, 15, 16 & 17).

2. *A. philippinensis*.
Recorded from Bengal,
Assam, North-eastern
parts of Bihar, and west
coast of India.

- (a) Internal and external clypeal hairs as in *annularis*.
- (b) Posterior clypeal hair has 7-9 branches arising from the root without a stalk.
- (c) Internal sutural hair may have 2-4 branches.
- (d) Abdominal palmate hair on segment I well developed. (Figs. 48 & 49).

3. *A. pallidus*.
Found mostly in Central
Provinces and Orissa.

- (a) Internal and external clypeal hairs as in *annularis*.
- (b) Posterior clypeal hair with 3-5 branches at the end of a short stalk.
- (c) Internal sutural hair has dichotomous branching consisting of about 5 branches.
- (d) Abdominal palmate hair on segment I well developed. (Figs. 50 & 51).

4. *A. jamesi*.
Found mainly in Bengal
and Assam.

Closely resembles *A. annularis* from which it can be easily separated by palmate hair on abdominal segment I, which is well developed in *annularis* but is not so in *jamesi*. (Figs. 17 & 19).

5. *A. ramsayi*.
Recorded from Bengal,
Assam, Orissa and
Ceylon.

- (a) Both internal and external clypeal hairs have conspicuous spine-like side hairs.
- (b) Internal clypeal hair is very long and the terminal part vanishes away during focussing and can not be followed.

- (c) Posterior hair long and simple.
- (d) Internal sutural hair long and simple.
- (e) Palmate hairs developed on abdominal segments 3-7.
- (f) The filament of abdominal palmate hairs poorly developed and the end pointed. (Figs. 20 & 21).

6. *A. splendidus*.
Has a wide distribution
in India.

- (a) Both internal and external clypeal hairs prominently frayed.
 - (b) Posterior hair long and simple.
 - (c) Internal sutural hair long and with about three branches.
 - (d) Palmate hairs on abdominal segment I undeveloped but well developed on segments 3-7, and the filaments are short and not sharp pointed.
- (*A. splendidus* bears many points of resemblance to *A. ramsayi*). (Figs. 22 & 23).

GROUP VII.

Chief characters.

Internal clypeal hairs simple. (Fig. 35).

1. *A. subpictus*.
Widely distributed in
India.

- (a) Internal, external, and posterior clypeal hairs long and simple.
- (b) External sutural hair has more than three branches arising from the end of the stalk.
- (c) Metathoracic palmate hair undeveloped.
- (d) Abdominal palmate hairs have long filaments. (Figs. 35, 36 & 41).

2. *A. culicifacies*.
Distributed all over India
and Ceylon and found
also at high altitudes in
Kashmir.

Closely resemble *A. subpictus* except that the metathoracic palmate hairs are well developed. Inner submedian prothoracic hair has a dark root. (Figs. 40 & 47).

3. *A. vagus*.
Confined to Eastern and
Southern India.

- (a) Internal clypeal hair long and simple.
- (b) External hair less than one-third the length of the internal hair and simple.
- (c) Posterior hair short like anterior external hair and placed between the internal clypeal hairs.
- (d) Metathoracic palmate hair not developed.
- (e) Abdominal palmate hairs have long filaments. (Fig. 37).

4. *A. majidi*.
Distribution limited to Darjeeling and Jalpaiguri Districts in Bengal, and Mysore and Malabar districts in Southern India.
- (a) Clypeal hairs like those of *A. subpictus* except that the internal clypeal hairs are stouter and are exceptionally long.
(b) Metathoracic palmate hair well developed.
(c) Filament of abdominal palmate hairs short.
5. *A. sundaicus*.
A brackish water species and found near sea coast.
- Closely resemble *A. subpictus* except that hair No. 5 on mesothorax has usually three branches arising from the end of a short stalk.
(*A. subpictus* may have the same number of branches but they always arise from the end of a comparatively long stalk). (Figs. 42, 43 & 44).
6. *A. stephensi*.
Found generally in large cities in India.
- Closely resemble *A. subpictus* except that the external sutural hair has 2-3 branches at the end of a long stem. (Figs. 52 & 53).
(Internal clypeal hairs may occasionally show minute frayings).
7. *A. multicolor*.
A desert species and found in Baluchistan.
- Clypeal hairs resemble *A. subpictus*. The metathoracic palmate hair is also undifferentiated. Its distribution, habitat and the presence of characteristic head spots must be taken into consideration in differentiating this species from *A. subpictus*.
8. *A. turkhudi*.
Recorded from the North-Western and Central parts of India.
- (a) All clypeal hairs are simple but the posterior hair is abnormally long.
(b) Abdominal palmate hairs undeveloped on segments 1-3.
(c) The filaments of abdominal palmate hairs short. (Figs. 38 & 39).
9. *A. dthali*.
Baluchistan, North-Western India, Dehra Dun.
- Difficult to distinguish from *A. culicifacies*.
10. *A. sergenti*.
North-Western India.
- Similar to *A. dthali*.

Key to the identification of mature Anopheleline larvae.

Group 1.

Internal clypeal hairs long and simple.

Internal hairs branched into 2 or more branches.

A. aitkeni and varieties.

External hair heavily and characteristically branched.

External hair dichotomously branched (8-10 branches).

A. umbrosus.

External hair simple.

Inner submedian thoracic hair branched.

A. barbirostris.

Inner submedian thoracic hair simple.

A. hyrcanus var. *nigerrimus*.

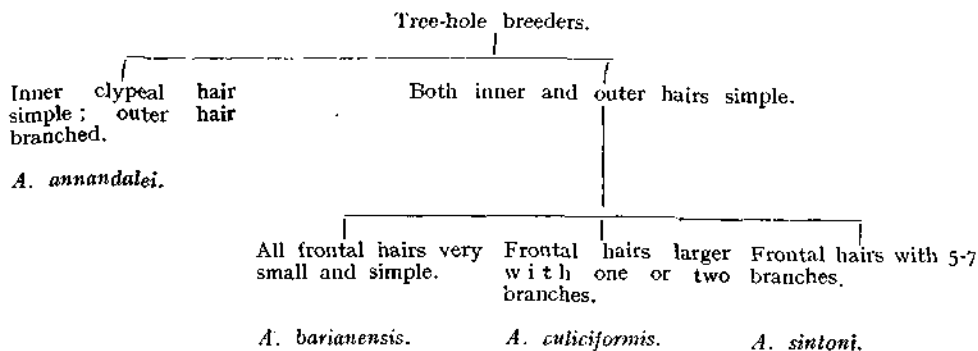
Abdominal palmate hairs developed on abdominal segments 2-7.

A. lindesayi.

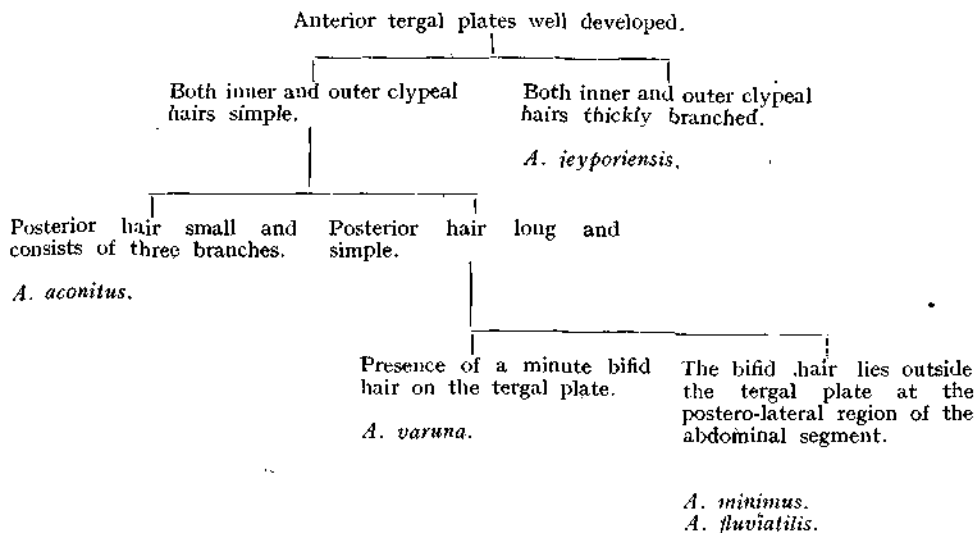
Abdominal palmate hairs developed on abdominal segments 3-7.

A. gigas.

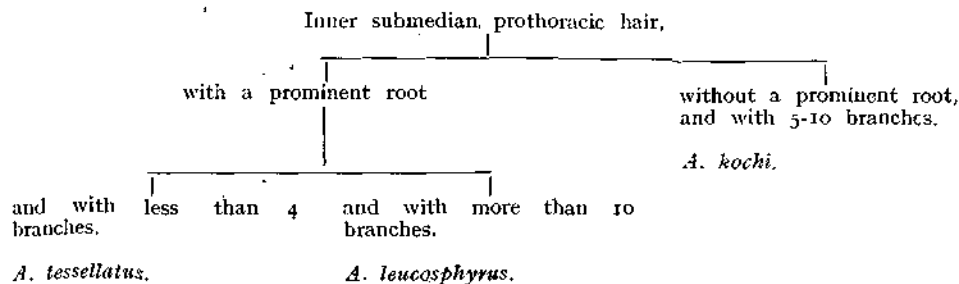
Group II.



Group III.



Group IV.



Group V.

Internal clypeal hair finely frayed.

External hair also finely frayed. Metathoracic palmate hair not differentiated.

Filaments of abdominal palmate hairs long.

A. maculatus.

Filaments of abdominal palmate hairs absent and the tip is blunt.

A. karwari.

External clypeal hair simple. Metathoracic palmate hair differentiated.

External clypeal hair has some long fine branches at the end of the stalk. Posterior hair has a short stalk and consists of 2-3 twigs.

A. pulcherrimus.

External clypeal hair simple. Posterior hair long and simple.

A. moghulensis.
A. superpictus.

N.B.—*A. superpictus* and *A. moghulensis* can be differentiated by the spots on the head or fronto-clypeus. This can be learnt from experience.

Group VI.

Both internal and external clypeal hairs thickly branched.

Branching of external hair is very heavy and is of a characteristic type.

External hair has side branches resembling thorns. Posterior clypeal hair long and simple.

Palmate hair on abdominal segment I undeveloped.

Palmate hair on abdominal segment I developed.

Internal sutural hair long and with about 3 branches.

Internal sutural hair long and simple.

A. jamesi.

Filaments of abdominal palmate hairs short and not sharp pointed.

Filaments of abdominal palmate hairs short but sharp pointed.

A. splendidus.*A. ramsayi*.

Posterior clypeal hair has over 7 branches arising from a root without a stalk.

Posterior hair has less than 5 branches.

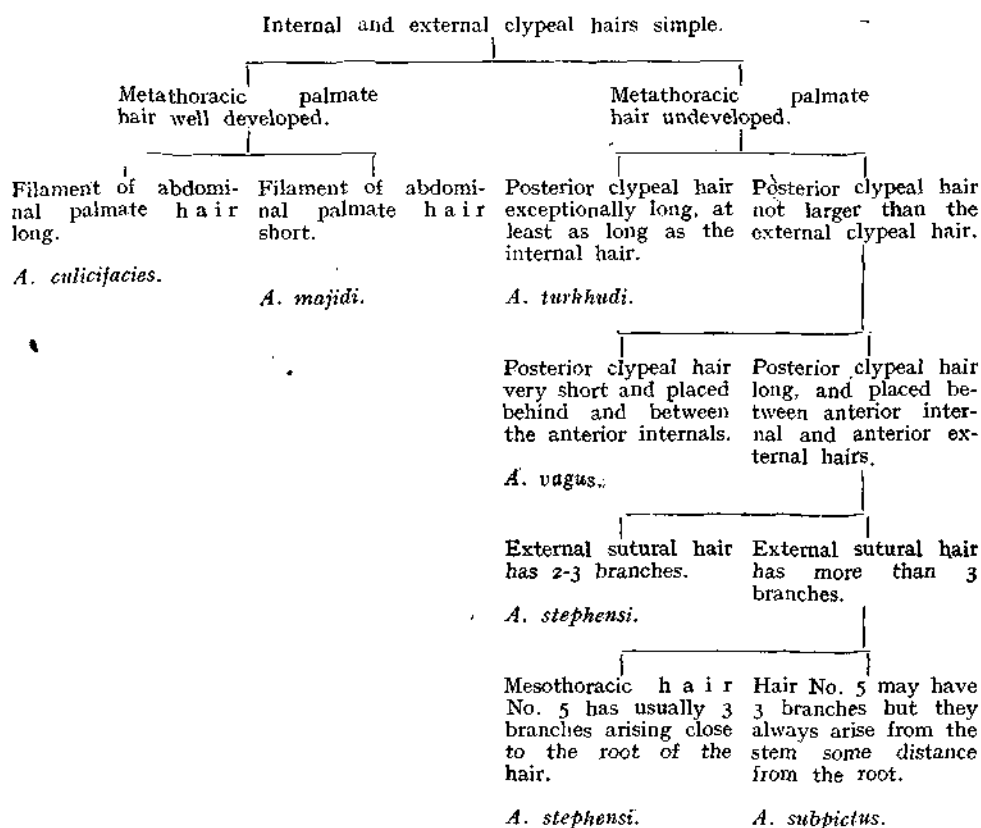
A. philippinensis.

Internal sutural hair long and simple.

Internal sutural hair has a dichotomous branching consisting of about 5 branches.

A. annularis.*A. pallidus*.

Group VII.



N.B.—(a) It is not easy to distinguish *A. multicolor* from *A. subpictus*. However, the former is a desert species and possesses certain spots on the head by which the two can be separated.

(b) It is also difficult to distinguish *A. dthali* and *A. sergenti* from *A. culicifacies*.

(c) *A. maculatus* can not be separated from *A. theobaldi* and *A. willmori* unless their distribution and habitat are known.

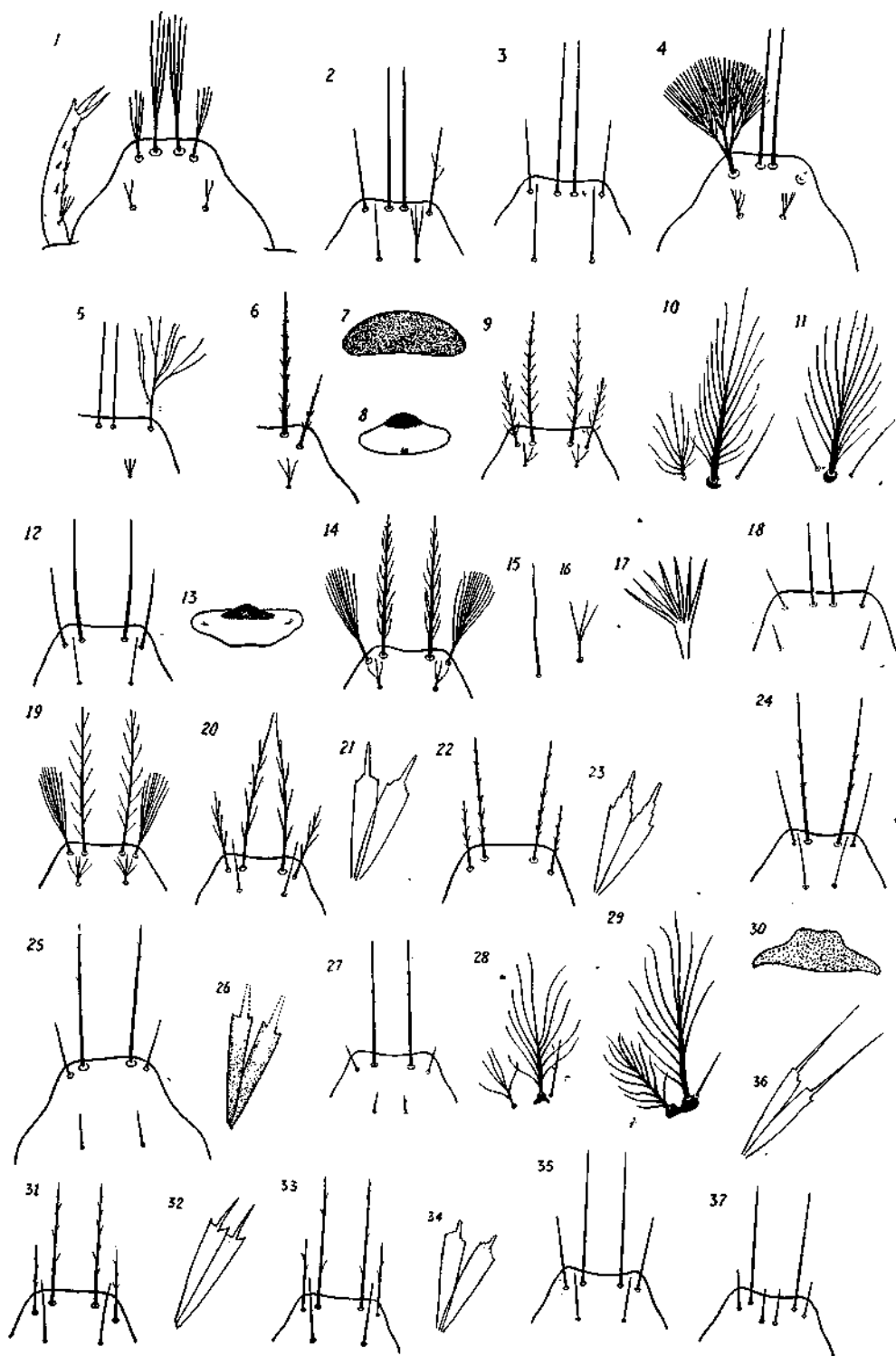


Fig. 47

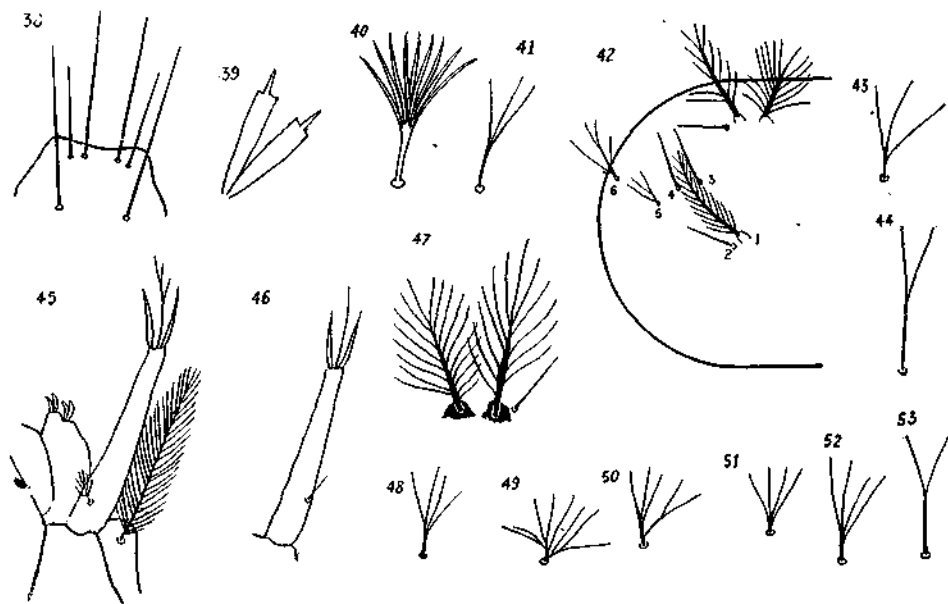


Fig. 48.

1. Antennal hair and clypeal hairs of *A. aitheni* var. *bengalensis*. 2. Clypeal hairs of *A. gigas*. 3. Clypeal hairs of *A. lindesayi*. 4. Clypeal hairs of *A. hyrcanus*. 5. Clypeal hairs of *A. umbrosus*. 6. Clypeal hairs of *A. aconitus*. 7. Anterior tergal plate of *A. jeyporiensis*. 8. Anterior tergal plate of *A. aconitus* and *A. minimus*. 9. Clypeal hairs of *A. jeyporiensis*. 10. Submedian prothoracic hairs of *A. barbirostris*. 11. Submedian prothoracic hairs of *A. hyrcanus* var. *nigerrimus*. 12. Clypeal hairs of *A. minimus*. 13. Anterior tergal plate of *A. varuna*. 14. Clypeal hairs of *A. annularis*. 15. Internal sutural hair of *A. annularis*. 16. Posterior clypeal hair of *A. annularis*. 17. Palmate hair on first abdominal segment of *A. annularis*. 18. Clypeal hairs of *A. barianensis*. 19. Clypeal hairs of *A. jamesi*. 20. Clypeal hairs of *A. ramsayi*. 21. Mid-abdominal palmate hair of *A. ramsayi*. 22. Clypeal hairs of *A. splendidus*. 23. Mid-abdominal palmate hair of *A. splendidus*. 24. Clypeal hairs of *A. moghulensis*. 25. Clypeal hairs of *A. moghulensis*. 25. Clypeal hairs of *A. kochi*. 26. Mid-abdominal palmate hair of *A. kochi*. 27. Clypeal hairs of *A. tessellatus*. 28. Submedian prothoracic hairs of *A. tessellatus*. 29. Submedian prothoracic hairs of *A. leucosphyrus*. 30. Mid-abdominal anterior tergal plate of *A. leucosphyrus*. 31. Clypeal hairs of *A. maculatus*. 32. Mid-abdominal palmate hair of *A. maculatus*. 33. Clypeal hairs of *A. harwari*. 34. Mid-abdominal palmate hair of *A. harwari*. 35. Clypeal hairs of *A. subpictus*. 36. Mid-abdominal palmate hair of *A. subpictus*. 37. Clypeal hairs of *A. vagus*. 38. Clypeal hairs of *A. turkhudi*. 39. Mid-abdominal palmate hair of *A. turkhudi*. 40. Metathoracic palmate hair of *A. culcifacies*. 41. Metathoracic palmate hair of *A. subpictus*. 42. Showing the position of mesothoracic hair no 5. 43. Mesothoracic hair no 5 of *A. sundaicus*. 44. Mesothoracic hair no 5 of *A. subpictus*. 45. Antennal hair of *A. aitheni*. 46. Antennal hair of *A. barianensis*. 47. Submedian prothoracic hairs of *A. culcifacies*. 48. Internal sutural hair of *A. philippinensis*. 49. Posterior clypeal hair of *A. philippinensis*. 50. Internal sutural hair of *A. pallidus*. 51. Posterior clypeal hair of *A. pallidus*. 52. External sutural hair of *A. subpictus*. 53. External sutural hair of *A. stephensi*.

MALARIA CONTROL

This is classified under the following headings ; (I) Personal prophylaxis. (II) Destruction of larvae. (III) Destruction of adult mosquitoes. (IV) Drainage. (V) Drug prophylaxis. (VI) Cattle prophylaxis. (VII) Naturalistic method.

(I) PERSONAL PROPHYLAXIS.

(a) Use of mosquito nets. Nets should be neither too small nor too large. When too large, they cannot be properly tucked under the mattress. When too small, the arms and legs may rest against the net ; these parts are liable to be bitten by mosquitoes. Nothing coarser than a mesh of 16 per inch is effective for protection against *Anopheles*.

(b) Use of fans, trousers, boots etc.

(c) Repellents or culicifuges.

Preparations containing vaseline are not suitable for use in the tropics especially during the rainy season. Fluid preparations are therefore recommended, and as far as possible should be freshly prepared.

The efficacy of a culicifuge is entirely dependent on its volatilization and this is greatly helped by a breeze. After a certain length of time its effect gradually fades and the application must therefore be repeated. It should be applied liberally on the exposed parts of the body such as the neck, hands, legs etc.

Among a large number of culicifuges which are reputed to possess such properties, the following are useful and the duration of protection obtained against the bites of *Aëd. aegypti* and *Armigeres obturbans* as observed in the laboratory is shown against them (Roy, Ghosh and Chopra, 1942).

	<i>Aëd. aegypti</i>	<i>Arm. obturbans</i>
(i) "Bamber oil": Oil of citronella 1½ parts, liquid paraffin 1 part, cocoanut oil 2 parts, carbolic acid 1 per cent.	30 min.	75 min.
(ii) Citronella oil:	45 min.	55 min.
(iii) Lemon grass oil:	1 hr.	1 hr.
(iv) Macnay's fluid: Concentrated extract of pyrethrum ½ oz., Castor oil 4 oz., Citronella oil 5 drops.	more than 300 min.	more than 300 min.
(v) Lemon grass oil, 10 parts. Concentrated extract of pyrethrum suitably diluted in kerosene 20 parts. Cocoanut oil 70 parts.	2 hr. 40 min.	2 hr. 52 min.
(d) Screening of houses.		

All doors, windows and other apertures should be screened. Screens of galvanised iron are not durable under coastal conditions. When made of copper,

they must be free from impurities. An aperture between 0.045 and 0.050 inch square will afford adequate protection against *A. culicifacies* in India (Mulligan and Majid, 1932), and *A. gambiae* and *A. funestus* in west Africa. (Davey and Gordon, 1938).

(e) Fumigation.

(i) Cresol vapour: it is slowly vaporised over a heated surface; after fumigation the room should remain closed for some time. Its vapour is irritating.

(ii) Pyrethrum: The inferior grades of pyrethrum are mixed with gum acacia and nitre and made into coils which, when burnt, effectively keep away all mosquitoes.

(II) DESTRUCTION OF LARVAE by Larvicides.

(a) Paris greening.

Paris green, the most widely used larvicide, is a double compound of the arsenite and acetate of copper. According to its formula it should contain 25.07 per cent of copper and 58.55 per cent of arsenious oxide. Commercial Paris green, as supplied for use as a larvicide, seldom contains these exact proportions of copper and arsenious oxide.

In the United States the law demands that Paris green must contain at least 50 per cent arsenious oxide. In this country where there is no such regulation, a sample containing less than 50 per cent should be rejected.

Paris green is a bright green compound, is insoluble in water but is soluble in dilute acids.

Roubaud (1920) was the first to advocate the use of powdered para-formaldehyde (trioxymethylene) to poison mosquito larvae.

Although arsenical insecticides have long been in use for the purpose of destroying caterpillars, its larvicidal properties were not known till Barber and Hayne (1921) found it extremely useful for destroying *Anopheles* larvae.

At present Paris green is the only larvicide available for use in breeding places that contain thick vertical vegetation. It is efficient and cheap, and in the proportion it is generally used it is thought to be harmless to man, domestic animals, and fish including *Gambusia*, though symptoms of chronic arsenical poisoning following the use of Paris green as an anopheline larvicide have been reported (Mackay, Buchanan and Sanderson, 1934). It is generally applied in 1 to 3 per cent strength after being diluted with any fine dust, such as road dust, soap stone powder, ash etc., road dust being the cheapest. The dust must be perfectly dry. Mixing should be thorough and is preferably done in a tight box or small barrel through which an iron pipe has been run diagonally and which can be revolved like a concrete mixer. Distribution should be either by hand or by a blower, the mixture being thrown in the air so that the wind will carry the dust cloud over the surface of the water. The distributors should take care to keep to windward of the dust cloud and to change their outer clothing and wash their hands before eating.

A litre of Paris green according to Hackett should control breeding over 10,000 sq. metres of surface or along 10 kilometres of bank. In the case of rice-fields in general one pound of Paris green per acre is a safe margin (Chalam, 1930).

When distributed over the surface of water the diluent used with the Paris green floats for a long time and helps to keep the latter in a floating state. Anopheline larvae which are surface feeders are quickly poisoned by the Paris green when the particles are swallowed by the larvae. It, however, has no effect on pupae nor on larvae of *Culex* and *Aedes* which are essentially bottom feeders. Its utility is curtailed in a region where there is a heavy rainfall.

Paris green when dusted directly over the open flowers of rice plants is liable to cause considerable harm to the paddy crop and its application should be restricted to the afternoon, *i.e.*, during the period when the flowers are closed (Covell, 1935). On the other hand, Rao and Sweet (1937) claimed that Paris green was totally innocuous to paddy flowers.

The interval between treatments should be shorter than the period required for *Anopheles* pupae to develop in that season.

It has been shown that Paris green may be distributed with kerosene without mixing with any dust; in this case the kerosene does not act as a larvicide but as a vehicle for spreading the Paris green and keeping it afloat. The Paris green is placed in a small quantity of kerosene; this is mixed with water and applied by spraying. The results are as good as those in which the Paris green is diluted with road dust (Barber, Rice and Mandekos, 1936). Russell, Knipe and Rao (1940) have advocated the preparation of a stock suspension consisting of Paris green, $2\frac{1}{2}$ lb., kerosene oil, $\frac{1}{2}$ gallon, castor oil, 1 oz., and the whites of 4 to 6 eggs. This is applied in a dilution of 1 oz. to 1 gallon of water from a knapsack oil-sprayer; 700 to 900 square feet can be treated with 25 c.c.m. of stock suspension. This method is particularly useful for transporting Paris green to the field.

For the destruction of *A. culicifacies* or *A. stephensi* which often find filter beds of water-works ideally suitable for breeding, Paris green is the only chemical larvicide which can be used with safety. It should be mixed with powdered soft stone and applied in 1 per cent strength. Particles of arsenic which settle down to the bottom are liable to become disintegrated after some time but though they lose some of their toxic properties, they nevertheless still remain theoretically poisonous. These, however, cannot penetrate the sand layer and are held back by the filter bed. Too frequent and unnecessary applications should be discouraged and only the required quantity should be used.

(b) Oiling.

The application of petroleum or its products to water surfaces for destroying all types of mosquito larvae and pupae is the most extensively used method of malaria prevention in the world to-day. It was first put into practice by Howard in the United States in 1892. It has been suggested that the oil gains entrance into the trachea through the spiracles of the larva and it quickly diffuses through the tracheal wall into the hæmocœle. It circulates in the body and attacks the ganglionic cells causing paralysis of the organs (Roy, Ghosh and Chopra, 1943).

The best penetrating oils are shown to be of a medium boiling range, not stable enough to be markedly viscous and not volatile enough to give that immediate dust cloud and to change their outer clothing and wash their hands before eating.

diately irritating effect which causes the larva to collapse its tracheæ and dive without receiving a dose of the oil (Murray, 1936).

According to Ramsay and Carpenter (1932) the lighter and more volatile the oil, the more readily it will penetrate into the spiracles.

However, up to the present no simple test can be applied to decide the efficacy of an oil for antilarval purposes, but a laboratory test will convey the necessary information in a short time.

Kerosene is the most commonly used oil and is generally mixed with a cheaper oil commonly known as crude oil. Pyrethrum extract or castor oil will markedly increase the spreading power of kerosene. 1 part of crude oil is added to 4 parts of kerosene before use.

The oil is applied by means of a knapsack sprayer so as to form a uniform film on the surface of the water. For slow-moving streams saw dust, waste cotton or waste jute is soaked with oil and placed in the stream. In the case of wells, petroleum has been recommended, and if applied at night, it leaves no trace in the morning.

In India, Malariol, a preparation of Burniah Oil Company, is extensively used. It is not only an efficient larvicide but also acts on grass and other vegetation, this being a great advantage in antimalarial campaigns. It also kills the pupae of all mosquitoes.

Oiling should be repeated once a week. The method of treating breeding places by oil has the great disadvantage of rendering the water unfit for drinking and of killing fish also. It cannot penetrate through aquatic vegetation especially grass. The oil film is liable to be broken up by wind thus interfering with its action.

Cost of malaria control.

The cost of malaria control by the use of chemical larvicides, e.g., oil, Paris green, is liable to vary in different places, the amount depending mostly on the local conditions. In the rural areas the *per capita* cost will be greater than in cities. However, in places where it has been effectively controlled it has been demonstrated that the cost of malaria to the community is far greater than the cost of effective control.

Sweet and Rao (1934) estimated the cost of malaria control in Mysore State by Paris green and larvicidal oil as varying from Rs. 2/- to Rs. 6/- per head per annum in villages of 500 to 2,000 populations and from annas -/12/- to Rs. 1/5/- in villages of 2,000 to 5,000 populations. They think that it may be possible to carry out efficient control in a compact area containing a number of large villages for annas -/8/- to Rs. 1/5/- per head. Compared with this the cost of successful antimalaria work in rural Singapore was about Rs. 3/- per head per annum.

(c) D. D. T.

This is not only a good insecticide but is also a good mosquito larvicide. It is insoluble in water but soluble in kerosene and is readily soluble in benzene, toluene etc. As far as possible only inferior grade kerosene should be used otherwise it will be necessary to expose the D.D.T. and kerosene mixture to the sun.

A 5 per cent solution has been successfully tried in the recent war for destroying mosquito larvae. It is sprayed by means of a knap-sack sprayer or distributed over the surface of water from an aeroplane.

(III) DESTRUCTION OF ADULT MOSQUITOES BY THE SPRAYING METHOD.

The control of malaria by killing adult *Anopheles* in dwelling houses has become a recognised procedure especially in the field during the time of war. Steudel (1911) first thought of the possibility of destroying anophelines in native huts in Africa when the mosquitoes rested in the house in a state of torpor during the dry season. Giemsa (1911, 1913) and Mühlens (1912) were able to destroy mosquitoes by spraying with an emulsion of pyrethrum, soap, and water and thus diminishing the spread of malaria. Since then its efficacy in malaria control has been clearly recognised. Covell and his co-workers (1938), who initiated this method of control in India, stated that with effective organisation and adequate supervision attempts to control malaria would be feasible at a *per capita* cost which would compare favourably with that of mosquito control by temporary anti-larval measures, especially in small isolated communities where other measures are economically impracticable, and where the vector species is known to rest in houses during the day time. The total cost of materials and labour for spray killing twice a week in Delhi worked out at roughly annas four *per capita* for the malaria season. In Madras, Russell and Knipe (1939) by spraying once a week found the *per capita* cost -/15/3 pies, and in Assam it was estimated at annas -/8/- (Viswanathan, 1941).

The reduction of malaria by spray killing has so far been successfully applied against *A. culicifacies* in the Punjab and Madras, *A. minimus* in Assam, and *A. fluviatilis* group of mosquitoes in the Singhbhum Hills. It has also proved successful against *A. philippinensis*.

There is always a remarkable reduction of the incidence of malaria especially among the newly born babies after spraying, though the spleen indices are little changed. There is no evidence that the potential malaria infectivity of a place can be reduced thereby. For spraying, a suitable sprayer preferably with a long nozzle should be used. Before the spraying is undertaken all doors and windows must be closed. An attempt should be made to dislodge mosquitoes from their resting places by shaking hangings and disturbing furniture, utensils etc. Five minutes spraying in each room must be considered quite sufficient and the spray is allowed to act for 30 minutes before the room is opened up. In cow-sheds, and thatched houses where it is difficult or impossible to close rooms satisfactorily, efforts should be made to obtain direct hits. Spraying on a systematic scale should be started before the commencement of the malaria season and continued throughout the malaria transmission period.

(IV) DRAINAGE.

The flow of surface water may be regulated by draining, damming etc. Drains may be exposed on the ground or laid under the surface. The latter method prevents the appearance of subsoil water on the surface as the water is carried along pipes laid under the ground.

(V) MASS DRUG PROPHYLAXIS.

It is now well established that the malarial sporozoite does not enter the red cells immediately after its introduction into the body but is thought to undergo multiplication in the endothelial cells. At this stage it is not acted upon by any drug whatsoever. It is therefore apparent that there is no drug which can render adequate protection to an individual against malarial infection. The anti-malarial drugs have therefore only therapeutic values. As some of them are endowed with the destruction of gametocytes, they can be used for gametocyte prophylaxis. Thus the sources from which the local mosquitoes may be infected are reduced. Mass drug prophylaxis is also used for protecting the population undergoing prophylactic treatment from the clinical manifestations of this disease. As soon as the administration of the drug is discontinued, the parasite and sporozoite rates quickly rise.

The views expressed in the 4th report of the Malaria Commission are as follows:

No prophylactic method, unless applied to disciplined communities under stringent supervision, is capable of attaining the desired objects. Very useful results can be obtained with daily doses of quinine (0.40 gm.) administered during the whole of the malaria season and even for a few weeks longer. This is also true of bi-weekly doses of atebirin (0.20 to 0.40 gm., per week) administered in certain conditions. The daily dose of 0.05 gm. of atebirin for prophylactic purposes has proved inadequate. Plasmoquine should always be distributed under direct medical control. It is particularly useful for reducing the number of gametocyte carriers and arresting the transmission of infection to the *Anopheles* especially in *P. falciparum* infections. It is more difficult to administer treatment to children, who are the reservoirs of gametocytes. The doses and forms of administration of atebirin and plasmoquine to children have not yet been finally determined.

(VI) CATTLE PROPHYLAXIS.

The chief factor in the epidemiology of malaria is the proportion of mosquitoes that succeed in biting human beings, and this factor must certainly be influenced by the presence of cattle. The close association with cattle does not always prevent a human epidemic of malaria but it is also true that a ring of cattle sheds on the outskirts of a village with dwelling houses in the centre would reduce the chances of malaria.

It has been clearly demonstrated by Walch (1932) that the anthropophilic index of a species of anopheline varies greatly in localities where cattle are present and where they are scarce.

(VII) NATURALISTIC MEASURES OF ANOPHELINE CONTROL.

Such measures of malaria control have been reported from various parts of the world. The measures are chemical, physical and biological. The most significant chemical measure is the alteration of the pH of the water by organic substances. Among the different chemical factors which are thought to have a direct relation with anopheline breeding are the albuminoid nitrogen, dissolved oxygen, oxidised nitrogen, ammoniacal nitrogen etc.

(a) *Fouling the water.*

The important vectors prefer moderately clean water for breeding, and pollution with sillage, sewage and industrial wastes of vegetable matters have been recommended to stop their breeding. Rao (1941) claims to have eliminated the breeding of *A. annularis*, the vector species in Khurda Road, by allowing sillage into rice fields in the dry season once a week, though this had the effect of increasing the breeding of non-carrier *Anopheles* and decreasing the breeding of carriers.

It was suggested by Williamson (1927) that foul water was in some way or other inhibitory to parasitic development in the adult such as *subpictus* and *vagus*. No difference, however, was noticed in this respect in mosquitoes bred out from water heavily charged with nitrogenous constituents and those from modified tap water. (Russell and Mohan, 1939).

(b) *Alteration of salt content of water.*

The alteration of salt content of the water has also been found useful. The breeding of brackish-water species, *A. maculipennis* var. *atroparvus*, has been checked in the coastal provinces in Holland by this process, its place being taken by *A.m.* var. *messae* where the water has been made definitely less saline. Similarly, by increasing the salinity of water by converting a brackish marsh at Durazzo, Albania, into a salt water lagoon, the breeding of *A. elutus* has been completely eliminated.

(c) *Physical measures.*

Physical measures are many, such as (i) removal of shelter of larvae by eliminating aquatic vegetation, deepening the edges of tanks and ponds, converting marsh into numerous small and tidy tanks stocked with fish and confining water into definite channels in which it can flow freely.

A. minimus breeding in many perennial rivers can be effectively controlled by merely removing the grass from the edge and exposing the bare edge to sunlight.

Such physical measures if carried out effectively will go a long way towards suppressing the breeding of anopheline larvae in general and not of any species in particular.

Other measures include the treatment with silt-laden water, sluicing, draining etc.

(d) *Shading.*

Shading is a measure extensively practised in Malaya in dealing with seepages which are a prolific source of *A. maculatus* in the hills. The realisation of the danger of indiscriminate clearing of jungle followed the observations of Strickland that hill streams shaded by jungle, favoured the breeding of only the *aikeni* group. This group are all non-vectors, and in places where the forest was removed, this species was soon replaced by *maculatus*. In the low lands, on the other hand, the breeding of *umbrosus*, which takes place in peaty marshes in virgin jungle, was eliminated by clearing the forest.

In Assam Strickland (1925) was the first to suggest shading the edges of perennial streams by growing jungle as a measure of controlling the breeding of *A. minimus*, whereas Ramsay (1931) was the first to demonstrate its practical

usefulness. The object is to ensure dense and complete shading so that no sunlight can reach the water surface ; this stops the growth of aquatic vegetation especially grass. This has also been successfully practised against *A. fluviatilis* in southern India.

The following plants have been recommended for this purpose. *Artemisia vulgaris*, *Adhatoda vasica*, *Tithonia diversiflora*, *Vitex negundo*, *duranta*, *lantana*, *hibiscus* etc.

In place of plants, edges of tanks have been successfully shaded by placing matting on the water.

(e) *Herbage packing.*

This method of malaria control aims at the suppression of the breeding of the malaria carriers by altering the quality of the water. It is well known that the malaria carriers, with the possible exception of *A. sundanicus*, prefer clean water, and some of them, exceptionally clean water. Williamson (1933, 1934) was the first to experiment with herbage packing in hill streams in Malaya against the breeding of *A. maculatus*. In herbage covered streams there is a definite succession of culicines, though anopheline breeding is arrested in the packed section. The ovipositing mosquitoes can, however, penetrate the herbage cover in large numbers and the method appears to act by biological means (Senior White, 1936).

Williamson's herbage packing has also been practised in India and the results have been as successful as in Malaya (Senior White, 1936 ; Covell and Harbagwan, 1939).

It is very important that green-cut vegetation be used and packed in successive layers.

(f) *Extension of agricultural activities.*

The extension of agricultural activities, e.g., by planting rice, will always lead to a change of the fauna and the betterment of the economic conditions of the people.

(g) *Biological control by fish.*

The employment of fish in the control of mosquitoes is nothing new. During the aquatic life of a mosquito, it is vulnerable to attacks by its enemies, among which fish may be considered as the most important. They are undoubtedly the cheapest, safest and surest means of preventing mosquito breeding. But their movement is greatly restricted in the presence of aquatic vegetation.

In selecting fish best suitable for employment as a larvicide, the natural habits of both fish and mosquito larvae must be taken into consideration.

Lebistes reticulatus, or 'millions', have been successfully employed in Barbados and *Gambusia affinis* in America and other places. In Java, both *A. sundanicus* near the coast, and *A. aconitus* in rice fields have been controlled cheaply and effectively by the scientific application of the knowledge of the feeding habits of certain fishes.

Fish may be placed into two categories such as the surface feeders and the bottom feeders. Among those that feed at the surface, *Haplochilus (Panchax)*

panchax and *Gambusia affinis*, both being carnivorous, are the most useful. The former is widely distributed in any natural collection of water in India. It is replaced by *H. lineatus* in the Peninsular part. *Gambusia affinis*, an imported

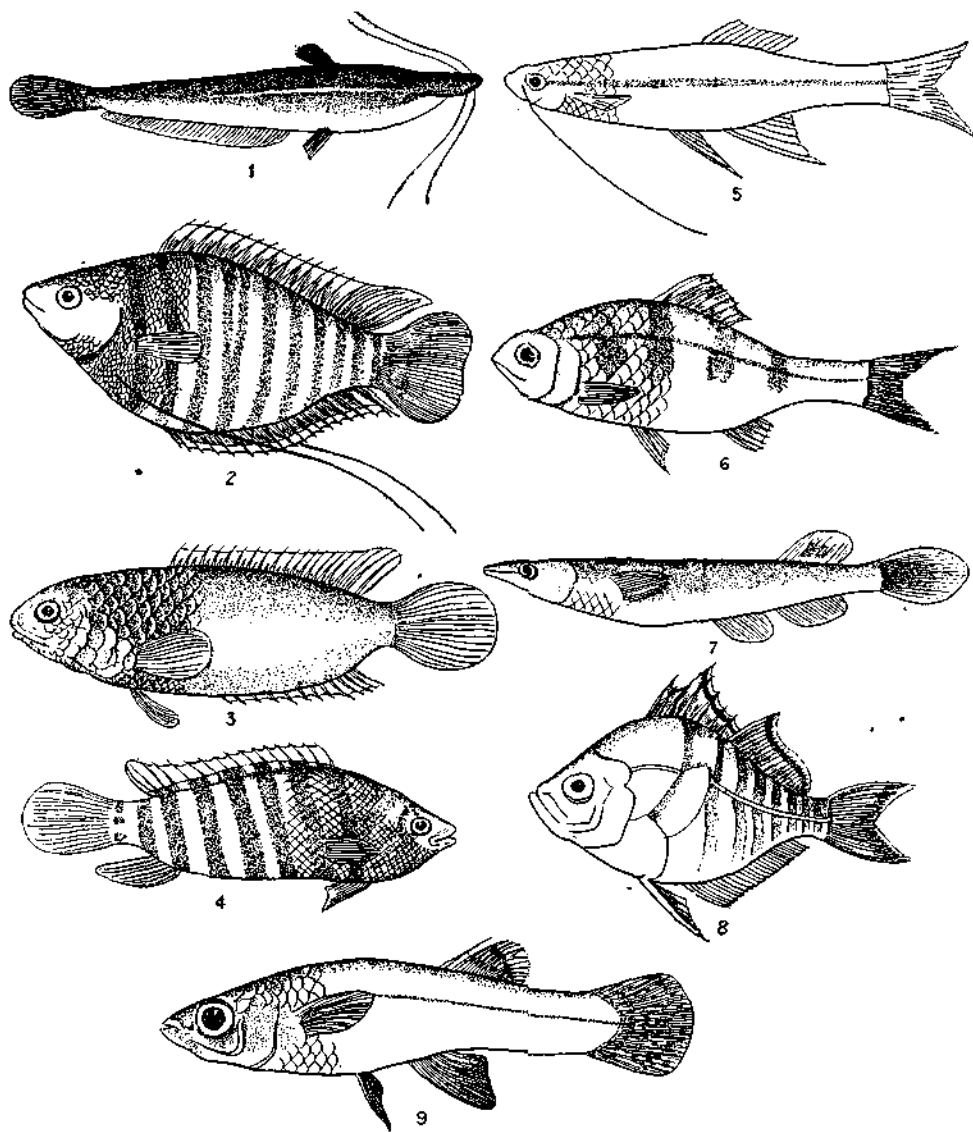


Fig. 49

Common larva-eating fish.

1. *Saccobranchnus fossilis*. 2. *Trichogaster (Colisa) fasciata*. 3. *Anabas scandens*.
4. *Badis badis*. 5. *Esomus danrica*. 6. *Barbus phutunio*. 7. *Panchax panchax*.
8. *Ambasis rangā*. 9. *Gambusia affinis*.

variety, devour mosquito eggs, larvae and pupae in large numbers. They are prolific breeders, are easily transported and are capable of withstanding a large range of temperature. They survive under very unfavourable conditions, even in

the smallest residuary puddles, and thrive not only in highly polluted but also in saline water. They readily succumb in the presence of any iron oxide or copper sulphate in water. They are viviparous, each female depositing 50—100 fully developed young ones at a time. When adequate food is not available the adult fish will prey on the young ones.

In India Russell and Jacob (1939) have met with good control not only of mosquito breeding but also of malaria by means of *G. affinis*. The experiments were carried out against *A. culicifacies* breeding in the sandy and scantily vegetated casuarina pits in the coastal areas of Madras. Sweet and Rao (1934) were able to control *stephensi* breeding in wells in Bangalore City by employing *Gambusia*.

For the treatment of mosquito larvae breeding in filter beds fish must be regarded as the most suitable. *Gambusia*, which prefers animal in place of vegetable food, are least likely to disturb the algal bed and even if they do so, this will not seriously derange the filtration arrangements.

It should, however, be known that fish do not particularly attack mosquito larvae in the presence of other types of food. Both Buxton (1922) in Palestine and Sen (1937) in Bengal failed to find Culicid larva in the alimentary canal of the fish dissected by them.

The bottom feeders such as *Trichogaster fasciatus*, *Badis badis*, *Barbus phutunio* and *Nuria danrica*, all natives of India, may be employed against the breeding of mosquitoes in a confined space. *Anabas scandens* and *Saccobranchus fossilis* are ideal for employment in wells.

It must be borne in mind that antilarval measures by oiling cannot be carried out in conjunction with the employment of fish, as the latter are quickly killed by oil.

In Java the method of malaria control by fish, as advocated by Walch and Schuurman (1929), has reached almost the level of perfection.

A marine fish *Chanos chanos* is cultivated in artificially constructed salt water ponds in Batavia (Java) in which *A. sundaicus* breed in association with floating algae consisting of *Enteromorpha*, *Chaetomorpha* and *Spirogyra*. The bottom of the ponds is laid dry for a couple of days at least once a month followed by filling up the pond with fresh sea water. During the draining period the fish remains inside ditches of the pond dug for the purpose. The top algae are killed by drying and the blue algae (Cyanophyceae) is thus encouraged to grow at the bottom. The fish live upon these Cyanophyceae. In addition, larvivorous fish such as *Haplochilus panchax* are released at the rate of 115 per acre; any larva that might be present are eaten.

In the inland areas fresh-water carp ponds are full of submerged water plants and vegetation and these breed anophelines such as *A. aconitus*. Walch's method of biological control consists of introducing in addition to the carp, a species of fish *Puntius javanicus*, which feeds exclusively on plants, as well as the larvivorous *panchax*. As a rule the edges of the pond are clean weeded.

It is therefore apparent that the chances of success of malaria and mosquito control in general by fish depend on certain conditions being fulfilled; among them are (i) absence of vegetation so that the fish may move about freely; (ii) presence

of the suitable type of fish ; (iii) absence of predators ; (iv) surface of water moderately clean ; this will ensure the minimum amount of food other than mosquito larvae for the fish ; (v) where enough food is not available, outside food has to be introduced for the fish.

TRIBE CULICINI

GENUS CULEX L.

These mosquitoes are important for the reason that a large number of them frequent dwelling houses and are terrible pests of man. At least one species,

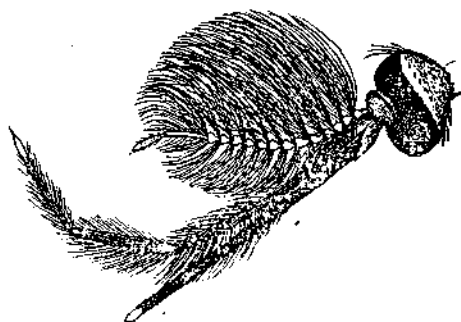


Fig. 50.
Culex head.

C. fatigans, has a wide distribution and has been proved to be the principal intermediate host of *Wuchereria bancrofti*.

Characters:—They are usually moderate-sized mosquitoes ; male palpi are longer than the proboscis and curved upwards at the extremities ; female palpi are quite short. The genus *Culex* is distinguished from all other mosquitoes by the possession of distinct pulvilli.

Eggs are laid in rafts and the larva has a long and narrow siphon tube.

Synopsis for the identification of the Indian species of *Culex* L with a pale ring on the proboscis in both sexes (from Barraud, 1923).

- | | |
|--|---------------------------|
| 1. Wings speckled with light and dark scales, or with patches of pale scales | 2 |
| Wings dark scaled | 4 |
| 2. Wings with patches or spots of white scales as in many species of <i>Anopheles</i> | 3 |
| Wings speckled all over with light and dark scales | <i>bitaeniorhynchus</i> . |
| Wings with a yellowish patch near the tip over the upper fork cell, rather large yellowish mosquito, with pale band on the proboscis occupying nearly two-thirds of the length | <i>epidesmus</i> . |
| 3. Third longitudinal vein with a pale area in the middle | <i>mimeticus</i> . |
| Third longitudinal vein entirely dark | <i>minutus</i> . |
| 4. Anterior two-thirds of mesonotum conspicuously whitish or pale greyish ochreous, posterior one-third dark | 5 |
| Anterior two-thirds of mesonotum not conspicuously paler than the posterior one-third | 7 |
| 5. Abdomen with basal bands, anterior two-thirds of mesonotum with white scales, small species | 6 |
| Abdomen with apical bands, anterior two-thirds of mesonotum with greyish or ochreous scales | <i>sinensis</i> . |
| 6. Tarsi with basal rings only, pale area of mesonotum produced posteriorly in four lines to scutellum, wing scales broad | <i>whitmorei</i> . |
| Tarsi with basal and apical rings, pale area of mesonotum not produced to scutellum, wing scales narrow | <i>gelidus</i> . |
| 7. Abdominal tergites with apical bands | <i>cornutus</i> . |
| Abdominal tergites with basal bands | 8 |

- | | | | | |
|-----|---|-----|-----|--|
| 8. | Mid and hind femora with a pale stripe anteriorly | ... | ... | <i>edwardsi</i> . |
| | Mid and hind femora not striped | ... | ... | 9 |
| 9. | Femora dark, speckled with pale scales | ... | ... | <i>sitiens</i> . |
| | Femora dark, without a speckling of pale scales | ... | ... | 10 |
| 10. | Mesonotum clothed uniformly with dark brown scales, tibiae without any pale stripe on the outside | ... | ... | <i>tritaeniorhynchus</i> . |
| | Mesonotum with brown and some lighter scales, mid and hind tibiae with a more or less distinct pale stripe on the outside | ... | ... | <i>vishnui</i> , <i>barraudi</i> ,
<i>whitei</i>
(separated by male hypopygium). |

C. fatigans : The most important species, *C. fatigans*, has no pale ring on the proboscis which is of uniform colour throughout its whole length.

Breeding habits.

C. bitaeniorhynchus : a common mosquito ; breeds in weedy pools, ponds and ditches.

C. sinensis : though widely distributed, it is never found in large numbers in any place.

C. sitiens : extremely common ; breeds in ground pools, rice fields, marshes etc.

C. tritaeniorhynchus : as widely spread as *vishnui* and has the same breeding habits.

C. whitmorei : breed in ground pools.

C. fuscocephalus : widely distributed ; breed in ground pools, rice fields etc.

C. mimeticus : a hill species.

C. barraudi : recorded from the Punjab and Western Himalaya

The culicines found commonly in cowsheds or in dwelling rooms are *Culex fatigans*, *C. vishnui*, *C. bitaeniorhynchus*, *C. gelidus*, and *C. sitiens* ; of these *fatigans*, *vishnui*, and *sitiens* are known to bite man.

CULEX FATIGANS L.

It is characterised by the absence of a distinct pale band or ring in the middle of the proboscis. It is a medium-sized brown species. The abdomen is provided with ochreous or yellowish basal bands ; pleurae pale brown and the venter of the abdomen pale.

Life history. This species is one of the commonest of Indian mosquitoes and is found everywhere in this country up to 6,000 ft. or more. It alone is responsible for the winter and spring plague of mosquitoes experienced almost all over India. It is essentially a domestic species and the larvae are found in localities inhabited by man.

The egg raft of *C. fatigans* is boat-shaped and may contain upwards of 200 eggs. The egg is ovoid, and dark in colour ; it floats with the narrow end pointed upwards and the broad end, which corresponds to the head of the larva, lies on the surface of water. To the broad end is attached a knob-like micropylar process

through which the sperm travels at the time of fertilisation of the ovum. Oviposition always occurs at night.

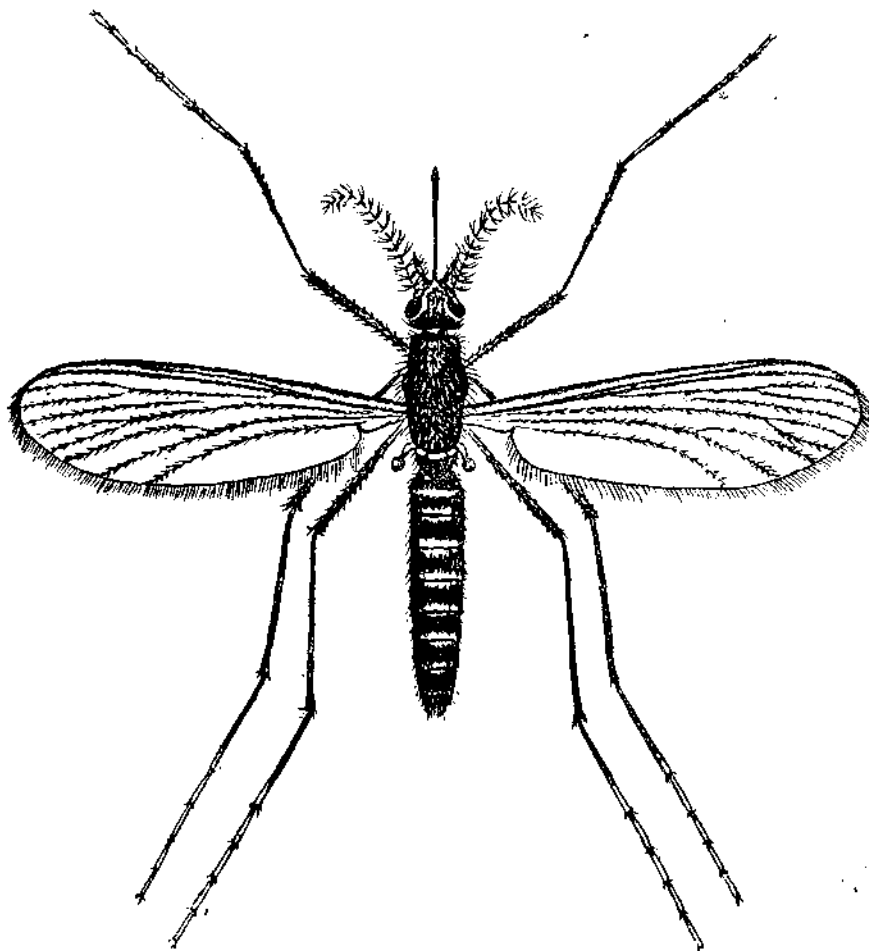


Fig. 51
Culex fatigans female.

The maturation of the ovum inside the ovary takes at least 72 hours following a blood meal and the earliest time eggs are deposited is at the end of four days after emergence (Roy and Majumdar, 1939).

The larva has a long and narrow siphon tube ; it is nearly four times as long as it is broad near its base. The antenna has a tuft of hairs at the junction of the proximal two-thirds and the distal third.

The peak of *Culex* breeding takes place in spring.

It breeds in almost any kind of water but where it breeds in prolific numbers it chooses water contaminated with sewage, *e.g.*, in cesspools, septic tanks, sewage farms etc. They also breed in ground pools, cement pits, drains etc. In British Guiana they have a fondness for rain-water containers. *C. fatigans* shows both

acidophilic and alkaliphilic tendencies (Mac Gregor, 1921 ; Buxton and Hopkins, 1927). Rain in the evening has an adverse effect on oviposition (Buxton and Hopkins, 1927). The larvae subsist on bacteria and other organic materials.

The period from the egg to the adult stage takes from 8-10 days during January in Calcutta. Its biting reflex is at its maximum at 81 per cent relative humidity (Mayne, 1930). This species is unable to stand wide variations of temperature and humidity. It avoids both high and low temperatures (Thomson, 1938). According to Gill (1921), a temperature of 80.6°F and a R.H. of 48 per cent is necessary if the adults are to live for 5 days. Majid and Sinton (1933) found 80 per cent R.H. the optimum for its longevity. According to Afridi *et al.* (1940), temperature is definitely correlated with the rise of the mosquito population ; a rise in the minimum and maximum temperature above 70°F and 100°F respectively, is associated with a steep fall in its number.

The meteorological conditions which influence the development of *Proteosoma* infection in

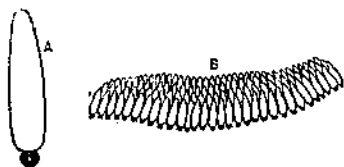


Fig. 52

A, An individual egg of *Culex fatigans* with micropylar process.

B, *Culex* egg raft.

C. fatigans are in certain respects different from those seen in plasmodial infection in *Anopheles* mosquitoes.

Thus Gill (1921) observed that with a relative humidity less than 40 per cent, *Culex* would not feed ; between 40 and 48 per cent, *Culex* fed but, if infected, died within 5 days. If the humidity was over 50 per cent, they fed freely and survived. Therefore *Proteosoma* can not be transmitted with a humidity of less than 48 per cent. At 104°F *C. fatigans* dies within 15 minutes whereas *Anopheles* remains full of life.

This mosquito is capable of dispersing over a wide area, and it is able to cause a nuisance at a distance of 1½ mile from the breeding area (Afridi and Majid, 1938).

The adult is generally reluctant to feed in the cage till three or four days have elapsed after its emergence. Mating takes place in captivity. It is most active towards dusk and during the first part of the night.

C. pipiens. It resembles *C. fatigans* very closely and from which it

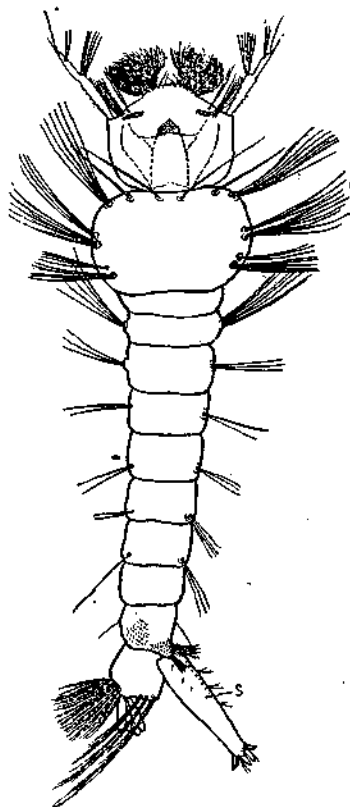


Fig. 53

Larva of *Culex fatigans*, s, Siphon tube.

can be separated with certainty by examination of the male genitalia. Another minor point of distinction is the venation. In *pipiens* there is the relatively greater length of the stem of the 2nd longitudinal vein.

C. pipiens has not yet been recorded from India though both *C. fatigans* and *C. pipiens* occur in China and in many other regions. It is a common mosquito in the northern latitudes and has recently been incriminated with *C. fatigans* in the transmission of *W. bancrofti* in China.

Two distinct biological races, autogenous and anautogenous, have been recognised by Roubaud (1929). They differ widely in their breeding, mating, and feeding characters.

In Europe and North America it appears to be the common vector of *Plasmodium relictum* of birds.

A significant difference has been noticed in the nutritive value of human and avian blood for egg production in this species. Those feeding on canary produce nearly twice the number of eggs than those fed on man (Woke, 1937).

Control.

Culex fatigans is a well-known insect pest of man especially during the late winter and spring. It is always difficult to convince any ordinary man that anti-*Anopheles* and anti-*Culex* measures are not the same and that the object of these measures are different. So long as *Culex* nuisance can not be reduced, it will be hard to obtain money for antimalaria work.

The control of *Culex fatigans* is not difficult but it is expensive. One thing that is required is to prevent the accumulation of sewage contaminated water anywhere within a distance of three miles from the outer perimeter of the locality. For this purpose a properly planned drainage system should be installed. No sewage farming should be allowed within the same distance. In other situations such as ponds, ditches or other collections of stagnant water, oil should be used. It may be pointed out that the ordinary mixture of kerosene and crude oil does not act so well when the water is grossly contaminated with organic matters. Under such conditions some pyrethrum extract should be added to this mixture just before use and sprayed with a knapsack sprayer. The same remark also applies to the use of a 5 per cent mixture of D.D.T. and kerosene. Proportionately a much larger quantity of this will be needed for controlling *Culex* breeding in foul water than in cleaner water. Though pyrethrum acts much more quickly on mosquito larvae, D.D.T. presents the advantage that within 24 hours of its application, a whitish scum appears on the surface; this lasts for 3-6 days. During this period no fresh deposition of eggs will be noticed. Where rapid action is desired, especially during the rainy season, some pyrethrum may be added to the D.D.T. if necessary. We have found in field experiments that a minimum of 5 per cent D.D.T. and kerosene mixture will be adequate for larvicidal purposes for a surface area of 8 sq. inch and in covered or semicovered spaces oviposition by either *Anopheles subpictus*, *Culex fatigans* or *Armigeres obturbans* can be prevented for 7-10 days; where the larvae are exposed to the direct action of the sun, larvicidal properties may be destroyed within 4-5 days.

Defective septic tanks or effluents from septic tanks should be properly inspected for they may be the source of prolific breeding of *Culex fatigans*.

In suitable industrial places foul water may be allowed to flow into a marsh situated at a distance. Waste sulphuric and other acids may be utilised for destroying the larvae and preventing any further breeding of this species.

To cope with a heavy influx of *Culex* mosquitoes in the house or in cowsheds, pyrethrum and kerosene spray is perhaps the only effective remedy. Compared with *Anopheles*, *Culex fatigans* is more resistant to the action of pyrethrum. It is for this reason that a room does not usually remain *Culex*-free for longer than two hours after spraying, whereas *Anopheles* mosquitoes are reluctant to enter such rooms within 4 to 6 hours. We are not in a position yet properly to assess the value of D.D.T. and kerosene in this respect though it is claimed that the residual effect after spraying will last for days together. So far as our experience goes, we are of opinion that such effects are noticed only during the time the wall remains soaked with the fluid and as soon as the kerosene has evaporated off, D.D.T. ceases to act. Nevertheless mosquitoes can freely enter and leave such rooms though they may not rest on the walls directly treated with the insecticidal fluid. It is for this reason that though it may be difficult to find many mosquitoes in the room both in the daytime and at night for two or three days after spraying, such measures, however, will not afford absolute protection against mosquito-borne diseases especially malaria and filariasis. It is only the mosquito nuisance which is diminished.

It has also been claimed that walls whitewashed with an emulsion of D.D.T. will serve as an effective antimosquito measure, the action lasting longer than after spraying. The emulsion is prepared by dissolving D.D.T. in a small amount of benzene and thereafter an emulsifying agent like soap is added. We cannot attach any real practical importance to such measures from the point of view of preventing mosquitoes from migrating into habitations. Such properties and many others which have been attributed to D.D.T. are, according to the present state of our experience, highly exaggerated.

GENUS AEDES (STEGOMYIA)

Mosquitoes of the genus *Aedes* are responsible for the transmission of the causative agents of two important diseases of man, dengue and yellow fever. A less important part is played when they carry *Dirofilaria immitis* and *D. repens* to dogs. Only one species is definitely known to carry the microfilariae of *Wuchereria bancrofti* in Fiji.

The adult mosquito is ornamented with silvery white scales all over its body.

They lay their eggs singly, and the larvae have a short and broad siphon tube. They are all bottom feeders and breed in small collections of water especially in artificial containers.

Characters: Colour black or blackish brown; body ornamented with silvery spots and markings; spiracular bristles absent; female palpi short, the palpi in the male are normally longer than the proboscis; the last two joints slender, upturned with very few hairs; pulvilli absent; front and middle claws of the female

have an extra tooth ; 8th abdominal segment of female retractile and in some cases hidden within the 7th.

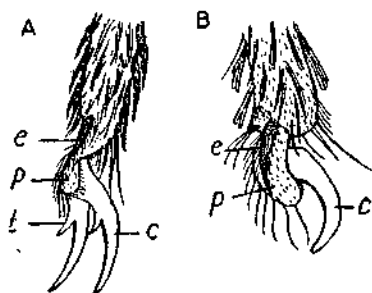


Fig 54
Front legs of (A) *Aedes aegypti* and
(B) *Culex fatigans*.
c, claw, e, empodium; p, pulvillus;
t, extra tooth.

Important species of this genus are, *Aedes aegypti*, *Aed. vittatus*, *Aed. albopictus*, and *Aed. scutellaris*.

Aedes aegypti L. is a domestic mosquito. It can be easily distinguished by the characteristic lyre-shaped markings on the thorax, while the larva has a pair of lateral hooks on each side of the thorax.

Aedes vittatus Bigot. (*Stegomyia sugens*)—It is not a domestic mosquito neither is it so common as *Aed. aegypti*. Mesonotum has four or six small round white spots. It is widely distributed in the Ethiopian and Oriental regions occurring as far south as Ceylon.

Aedes albopictus Skuse. is also an oriental species and possesses a single median silvery white stripe on the mesonotum and separate patches of white scales on the pleura of the thorax. Barraud (1934) gives tree-holes, bamboos, leaf-axils and only rarely artificial receptacles or rock-pools as their breeding sites. Sen (1935) found it breeding in broken glass jars and metal drums. Though it lives away from man, it bites freely whenever it gets the chance to do so.

Aedes scutellaris Wlk. (*Aedes variegatus*, Dol.)—is a widely-spread species in the Australasian region, and under its synonym of *Stegomyia pseudoscutellaris* is well known as the carrier of filaria in Fiji, and Polynesia in general. It has been recorded from the Andaman islands. In the Hawaiian islands the only representative of this group is *Aedes albopictus*. It is similar in habits to *Aed. aegypti*. It differs from *Aed. albopictus* in having three parallel white stripes on the pleura of the mesothorax and the white abdominal cross bands incomplete.

AEDES AEGYPTI L.

Aed. aegypti is the common tiger or yellow-fever mosquito and is widely distributed both in the tropical and subtropical regions. In colder countries it appears in the summer but with the advent of cold weather it disappears.

Adult—

Head: dark with a distinct double white median line ; palps black, white at the tip ; proboscis black.

Thorax: brown with characteristic lyre-shaped marking on the dorsum ; scutellum completely covered with silvery white scales ; pleura with several patches of brilliant white scales ; the first and second pair of legs with white bands on the tarsi, the hind pair with five white bands, the last joint being wholly white

Abdomen: dark with white bands on the bases of the segments and also laterally.

Egg. The egg is elongate, blackish in colour and the shell is highly chitinised. The outside is sparsely studded with minute hemispherical bodies of whitish secretory matter.

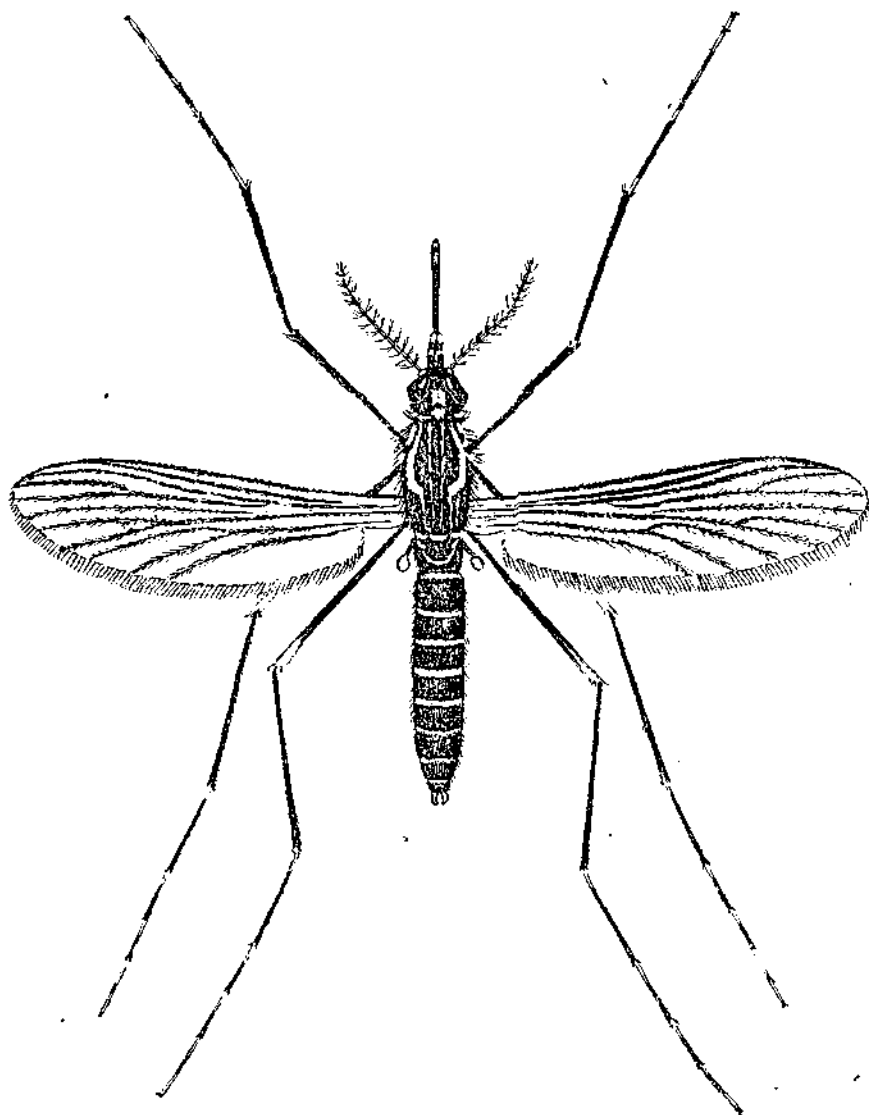


Fig. 55
Aedes aegypti female.

Larva. The larval head and thorax are broad ; antenna is almost bare except a single short hair on the shaft near the middle and a few delicate hairs at the apex. There is a pair of chitinous hooks on each side of the thorax. On each side of the 8th segment of the abdomen are the lateral combs arranged in a line, and composed of 8 to 10 serrated spines. The tracheal gills are long, and the siphon tube short and broad.

Pupa. The respiratory trumpets are shorter and broader than in *Anopheles*. In mature pupa the dark-coloured adult is visible through the pupal skin.

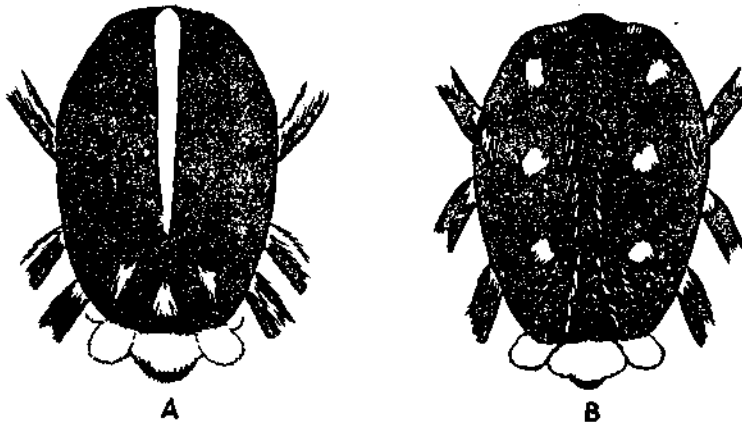


Fig. 56
A, *Aedes albopictus* ;
B, *Aedes vittatus*.

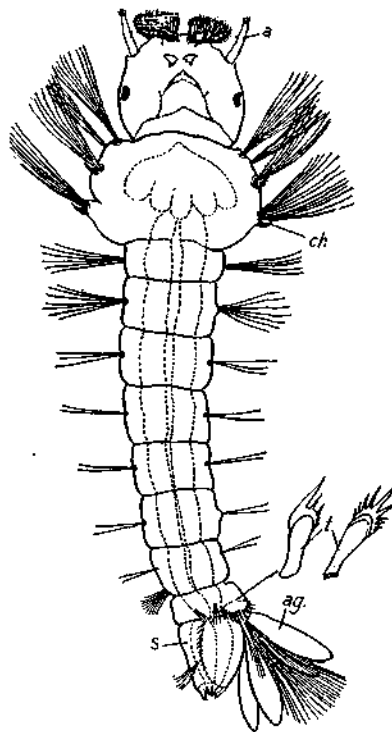


Fig. 58
Larva of *Aedes aegypti*.
ch, chitinous hook ;
ag, anal gill ;
s, siphon tube. .
a, antenna.



Fig. 57
Egg of *Aedes aegypti* (highly magnified).

Life history. The eggs are conspicuously dark objects and are always laid separately. The production of eggs has a direct bearing on the amount of blood ingested by the female. Buxton and Hopkins (1927) recorded the number of eggs per laying as maximum 37.3, mean 20.5 and minimum 8.3. The maximum number of eggs recorded by Roy is 83 (1936).

The eggs are deposited not only on the surface of the water but also along the sides of the containers above the water level and they adhere closely thereto. Eggs generally float on the surface but sometimes they sink to the bottom though this does not interfere with subsequent hatching. The eggs are very resistant to unfavourable environment and will hatch after storage for several months in a dry place.

It is not, however, a partly developed egg but a fully developed resisting larva enclosed in serosa and amnion. The larvae are ready to emerge under suitable conditions. They can thus be safely transported to distant places. For this purpose eggs should be picked up from water with a camel hair brush 40 hours after they have been deposited, and placed on dry filter paper.

As *Aedes aegypti* is a highly domesticated mosquito, its breeding places are never far from the abode of man. It seldom breeds in any natural collection of water but for reasons unknown it prefers small artificial collections. Its larvae are found in discarded tins, broken bottles, coconut shells, flower pots, flower vases, broken crockery, earthen pots, cut ends of bamboo, tree holes, rock holes, roof gutters of houses, choked drains, ant-guards, water cisterns, fire buckets, drinking water pitchers, barrels, leaf axils of pineapple and *Dressina* etc. Sometimes enormous numbers of larvae are found in rain-water collecting in country boats. A faintly saline water or a moderately foul water is not always rejected by the female for oviposition though this species is regarded as a clean water breeder showing a decided preference for rain water. It should therefore be borne in mind that a very small quantity of water from a tea-spoonful upwards may suffice as a breeding place for *Aedes*. It is particularly attracted to hay infusion and Buxton and Hopkins (1925) thought that the larvae might be destroyed by poisoning the infusion with arsenious oxide or copper sulphate and thereby check its breeding.

In the tropics the eggs generally hatch within two days after deposition. When covered by frost they retain their vitality for a long time.

Atkin and Bacot (1917) demonstrated that the presence of bacteria, yeasts and less definitely moulds, exerts a stimulus which causes eggs to hatch. This stimulus is less powerful if killed cultures or sterile filtration and extracts of bacteria and yeasts are used though it is possible to rear larvae under sterile conditions.

The larva has a characteristic worm-like movement and is a bottom feeder. What it actually feeds upon is not known but it is apparent that it can sustain itself on solids dissolved in the water though in the laboratory it greedily ingests solid particles. The retardation of the natural development of larvae of *Aed. aegypti* in the absence of micro-organisms has been noticed by Hinman (1932). They will grow, however, when autoclaved liver extract and yeast is added (Trager 1935). Similar observations have been made by Rozeboom (1934). The larvae grow very rapidly during the rainy season especially when they are kept agitated by rain drops. The larva is resistant to immersion and can be resuscitated after 2 hours' submersion. The larval life under the most favourable conditions lasts for 4 days.

The pupae of *Aed. aegypti* are not able to descend unharmed to such great depths as the larvae. They usually descend only an inch or two whereas the *Aedes* larva can go down even to 5 feet. (Macfie, 1923).

Sunlight is not at all essential for the development of *Aed. aegypti* (Jobling, 1937).

The adult female is a vicious biter and will persistently haunt man. They are apparently fearless. In presence of man it will never feed on any other animal. It is characteristically a day biter taking blood between day light and dusk but

when no blood is available in the day, it will bite at night. It can easily bite through thin socks and shirts.

Egg formation has not been known to occur without a suitable amount of blood feed. Fertilised ova may sometimes lie latent in the body for some time. The female lays her eggs on the fourth night after the blood feed and only one blood meal is required for laying.

Oviposition will not take place in a solution of common salt in 2 per cent strength and if eggs are laid at all, the larvae are readily killed (Macfie, 1921).

The adult mosquito definitely prefers dark and black material on which to rest.

It is a strictly domestic mosquito and is incapable of flying any great distance at one time. Its range of flight is probably restricted to 50 yards.

Both males and females can be sustained on honey, raisin, sugar, banana etc., for a long time extending to several months. The female lives longer than the male.

In captivity pairing takes place about 12 hours after emergence from the pupa and is followed by a meal of blood. Under certain laboratory conditions the female feeds readily on frog and turtle and produces viable eggs; blood from different species of vertebrates may differ in its effect on the egg production (Woke, 1937).

Hibernation does not take place in this species.

They can be easily transported in aircraft to distant places without producing any ill-effect to them (Hicks and Dewanchand, 1936). Similar observations have been made in America and in the Belgian Congo. This species especially the blood-fed female will survive for 3 to 4 hours when it is subjected to a pressure of 27 mm. of Hg. It is now fully realised that aerial transport involves new risks of introducing diseases into areas previously exempt from them, and of these yellow fever is the most feared.

It is carried from one place to another and even from one country to another in steamers, aeroplanes, railway trains and country boats. It is the most common mosquito found in ships and freely breeds in country boats and steamers.

The larvae of *Aedes aegypti* are particularly liable to be attacked by the gregarine parasite, *Lankesteria culicis* Ross.

Disease Relationship.

It is the most important and perhaps the only mosquito concerned in the transmission of dengue and yellow fever. The microfilariae of *Wuchereria bancrofti* do not develop in this species (Rao and Iyengar, 1932).

Dengue: *Aedes aegypti* has been proved by experimental transmission to be the carrier of dengue in Australia by Bancroft (1906) and by Cleland, Bradley and Mac Donald (1916), in Texas by Chandler and Rice (1923), and in the Philippines by Siler, Hall and Hitchens (1926). Experimental transmission has been attempted with other mosquitoes and though success has been claimed with *Culex fatigans*, such findings have not been confirmed. In Japan positive results have been obtained with *Aedes albopictus* and *Armigeres obturbans*.

The blood of a dengue patient is usually infective to the mosquito only during the first three days of the disease. The incubation period for the development of

the virus in *Aed. aegypti* is from 11 days to 14 days, and once infected, the infection lasts during the remainder of its life. There is no hereditary transmission of the virus. Schule (1928) later observed that *Aed. aegypti* may transmit dengue as early as the 8th day after the ingestion of the virus, and suggested that the rapidity of maturation of the virus in the mosquito may be influenced by temperature.

Yellow fever: Although Davis (1933), claimed to have succeeded in transmitting yellow fever virus by *Culex fatigans*, yet the chief mosquito vector is definitely *Aed. aegypti* and this was known until recently to be the only vector of this disease; so too was man believed to be the only animal host of the virus and not until 1927 was it demonstrated that the disease could be given to monkeys, *Silenus rhesus* and *S. sinicus*, with fatal effects (Stokes *et al.*, 1928).

Two different types of yellow fever have now been clearly recognised. These are the urban type existing in Africa and the rural type found in Brazil. It has been demonstrated in South America that yellow fever can exist and be transmitted in epidemic form in the absence of *Aed. aegypti* by two species of jungle mosquitoes, *Aedes leucoceloenus* and *Haemagogus capricorni*, as both these mosquitoes have been found infected in nature.

The infective material of yellow fever circulates in the blood of the patient for the first three days and during this time mosquitoes feeding on them become infected. It takes about 12 days before the mosquito is able to propagate the disease to non-immunes. The incubation period of yellow fever in mosquitoes depends to a considerable extent on the temperature. If kept at tropical room temperature averaging 23.4°C, it is infective after 11 days, and at 28°C, 9 days. At low temperatures the incubation period may be indefinitely prolonged (Hindle, 1930).

The possibility of the introduction of yellow fever into the United States or in India has now been increased on account of the tremendous advance in rapid transport by aeroplanes. Either infected mosquitoes or yellow fever patients may be carried in this way and may start an epidemic in the above countries. Infected human beings are, however, more important than mosquitoes.

If ever the disease by chance enters this country the danger will be all the greater on account of (1) the wide distribution of *Aed. aegypti* (Barraud, 1928, 1934); the Indian strain of this mosquito is as susceptible to yellow fever as the African strain (Hindle, 1929); (2) both the two common brown monkeys of India, *Silenus rhesus* and *S. sinicus*, are susceptible to the virus of yellow fever; these animals are not only widely distributed in this country but in many places they are domestic animals; (3) the circulation of the virus in the peripheral blood of the monkey throughout the course of the disease (Hindle, 1932); monkeys infected with yellow fever virus generally die within 4 days; (4) the discovery that in addition to *Aedes aegypti*, other species of *Aedes*, *Aed. vittatus*, *Aed. albopictus*, and *Aed. scutellaris* also *Mansonioides uniformis* have been proved to be capable of transmitting the disease experimentally. *M. uniformis* has a wide distribution in India and bites man freely; (5) suitable climatic conditions; (6) the presence of both susceptible human and insect populations.

thoroughly and systematically searched for both adults and larvae which must be destroyed.

- (d) Vaccination of the crews of trains and aircraft flying between infected and non-infected areas when the two are contiguous has also been suggested.
- (e) The establishment of sanitary aerodromes in both infected and non-infected areas.

Methods of vaccination.

The person to be immunised is given a suspension of mouse-fixed yellow fever virus followed immediately by immune yellow fever serum from a recovered case of yellow fever or from a previously immunised person.

Various modifications of the principle have been used and the process results in giving the treated persons a solid immunity comparable with that conferred by an attack of the disease, the difference being that it lasts for only 2-3 years.

Sanitary aerodrome.

Proper sanitary control over aircraft is essential. Those working on international lines should depart from and arrive at certain authorised aerodromes where the requisite sanitary provisions will be maintained. A sanitary aerodrome should be provided with adequate personnel and apparatus necessary to undertake disinsectization. The aerodrome must be situated at an adequate distance from the nearest inhabited centre. The hospital and other buildings situated in the aerodrome area must be screened. Such sanitary aerodromes are called "anti-amaryl" aerodromes.

For the destruction of mosquitoes in aircraft, pyrethrum extract is recommended and an aqueous emulsion of the extract can be used if necessary.

GENUS MANSONIA

Subgenus *Mansonioides*.

This mosquito is readily recognised by its speckled wings and legs, the speckling being due to broad scales of both light and dark colour; many of these scales are asymmetrical; the palpi of the male are longer than the proboscis; tergite VIII of female has a row or patch of short tooth-like spines; claws of female simple.

The female readily attacks man and is at times very troublesome. All are swamp or tank breeders especially those thickly overgrown with vegetation. Two species, *Mansonioides uniformis* and *M. annulifera*, have been proved to be transmitters of *Wuchereria malayi* (Iyengar, 1938) in India, and in addition to *M. uniformis*, *Anopheles hyrcanus* var. *sinensis* has been noticed to act as a true intermediate host in China (Feng, 1934), also *Anopheles barbirostris* and many culicine species in Dutch East-Indies. It is remarkable that microfilariae of *W. malayi* will not develop in *Culex fatigans*. It is also worth noting that both *Microfilaria bancrofti* and *M. malayi* may occur in the same individual (Wenyon, 1928).

There are only 4 species recorded from India.

M. annulifera Theo. Colour yellowish brown; mesonotum marked with 4

or more distinct round white spots. Widely distributed in Bengal, Bihar and Orissa.

M. longipalpis van der Wulp. Distributed in eastern India. Mesonotum marked with 2 or 3 round white spots.

M. uniformis Theo. Common in most parts of India. Mesonotum marked with a pair of sub-lateral greenish stripes on a brown ground.

M. indiana Edw. Found in Assam, Bengal and southern India. Resembles *M. uniformis* but mesonotum dark brown and not marked with greenish stripes.

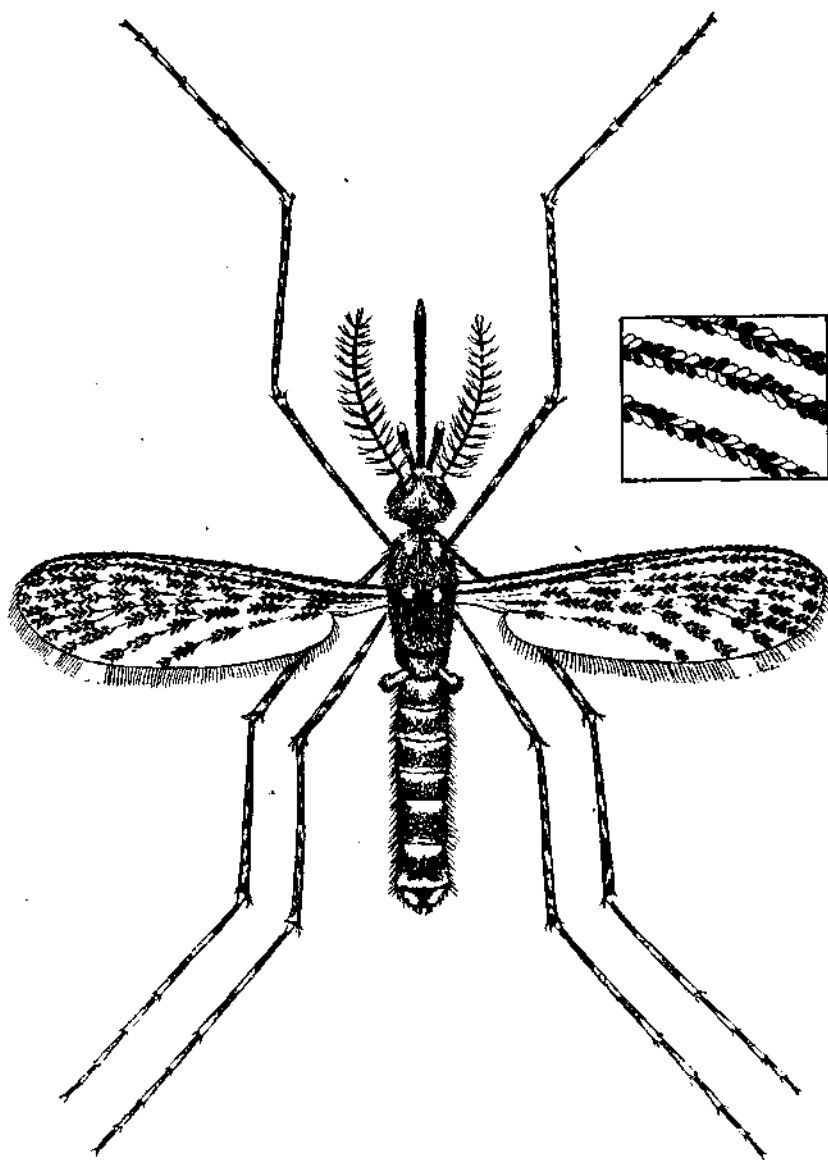


Fig. 59
Mansonioides uniformis female.

The characteristic feature about their breeding is that they depend on certain aquatic plants especially *Pistia stratiotes* for the supply of oxygen. Larvae and

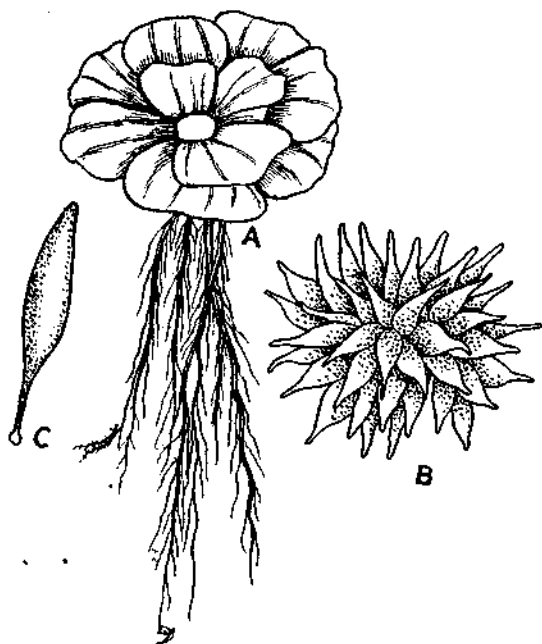


Fig. 60

A, *Pistia stratiotes* showing how larvæ and pupæ of *Mansonioides* mosquitoes remain attached to the roots.

B, Egg cluster of *Mansonioides*.

C, Individual egg.

pupæ do not come to the surface until shortly before the emergence of the mosquito. The larva is provided with a pair of long curved spines or hooks on the siphon tube and a kind of saw apparatus at the extreme tip. The larva remains anchored to the plant by means of the hooks and the saw apparatus is used for boring. The same mechanism also exists in the pupa.

The eggs are always laid in clusters on the under surface of the leaf.

These mosquitoes are the true intermediate hosts of *W. malayi*. Clearance of pistia will at once stop their breeding (Iyengar, 1937 ; Sweet and Pillai, 1937).

GENUS ARMIGERES

Though they are not known to convey any disease, they are fearless and intolerable biters and migrate in numbers in the dwelling houses.



Fig. 61
Pupa of *Mansonioides*.

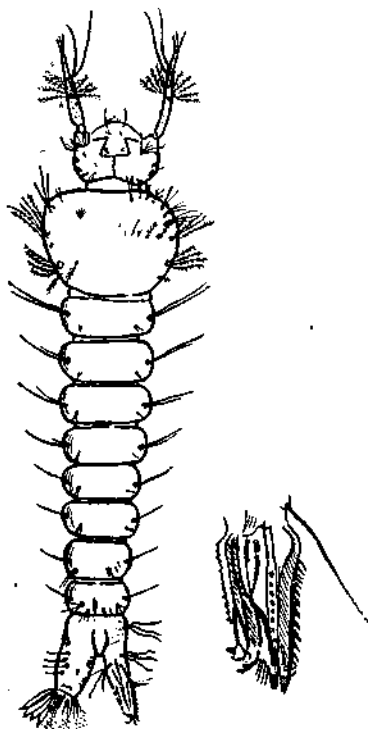


Fig. 62
Larva of *Mansonioides* showing the saw-like apparatus on the siphon tube.

They are distinguished from *Aedes* by their large size, absence of silvery white patches or spots on the mesonotum, and by the form of the proboscis, which is curved downwards at the tip, and distinctly flattened laterally; the female claws are toothed. They are fairly large mosquitoes with flat scales on vertex and scutellum.

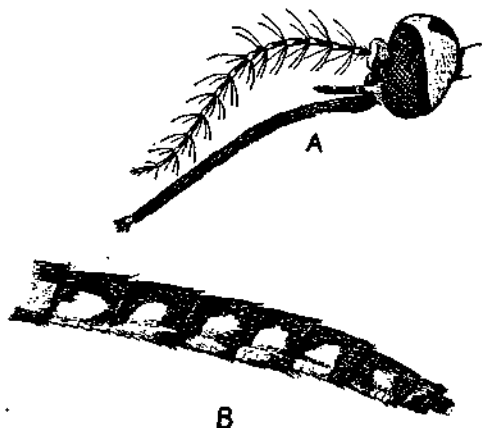


Fig. 63

A. *Armigeres* head and B. abdomen.

A. obturbans.

This species is widely distributed in India. It is a large, dark mosquito with a distinct pale border to the mesonotum, and with apical pale bands on the 3rd to the 6th abdominal sternites; these bands are usually wide and are all about the same width; palpi of the female about one-third the length of the proboscis; abdomen shows patches of white scales lateral-

ly; scutellar scales are dark; the tarsi are entirely dark.

Adults are intolerable biters. They migrate towards dusk and bite even in the day time. They breed in enormous numbers in small pools, and earthen drains grossly contaminated with urine. The earthen drains along which the blood from slaughter houses flow often contain millions of larvae. Another prolific breeding place is the vat used for soaking hides in the manufacture of leather. In these situations they breed throughout the year.

The larvae are predators and prey on other mosquito larvae. The tracheal gills are particularly well developed. The antennae are of uniform width and are almost bare. The siphon-tube is short and broad. The chitinous hooks on the 8th segment are arranged in a row as in *Aed. aegypti* but they have a different shape.

Both larvae and adults are particularly resistant to ordinary chemical larvicides but succumb quickly to D.D.T. or pyrethrum-kerosene mixtures.

A. obturbans has been successfully infected with *Plas. knowlesi* in India.

GENUS LUTZIA

The adult mosquitoes resemble the large species of *Culex* in general appearance and structure and in scaling. The adults are, however, distinguished from *Culex* by the possession of more numerous lower mesepimeral and proepimeral bristles.

L. fuscana Wied.: abdominal tergites 5 to 8 entirely yellow scaled or with broad apical bands; tergites 2 to 4 entirely dark or with very narrow apical bands. It is widely distributed chiefly in the plains.

The larvae are predators and also cannibalistic in habits, preying upon the larvae and pupae of other mosquitoes breeding together.

The mouth brushes of the larva are specialised and adapted for holding prey, each brush consisting of a number of strong curved rods with minute hairs along one side of the apical half ; the clypeus is armed with a number of processes.

The adult has been recorded as biting man.

L. raptor may be confused with *L. fuscana* but the former can be readily distinguished by the abdominal tergites which have all ochreous apical bands, the last few but little broader ; the outer side of the hind femur almost entirely pale scaled on the basal half.

It is found chiefly on the western side of India from the Punjab to Ceylon, and often with *L. fuscana*.

Mosquito and Filarial Diseases of Man.

C. fatigans is a proved carrier of the microfilaria of *Wuchereria bancrofti*. It is, however, refractory to that of *W. malayi* (Brug, 1927) which develops in *Mansonioides* species and in other species of culicines and in some anophelines in certain parts of China and Dutch East Indies.

The commonly accepted view regarding the mode of entry of larvae of *W. bancrofti* into the body cavity of the mosquito is that microfilariae from human blood enter the stomach of the mosquito and after casting their sheath penetrate through the wall of the stomach, enter the haemocoel and then find their way into the thoracic muscles. According to Iyengar (1936) the worms enter the haemocoel in a remarkably short time, frequently in less than half an hour after the infective meal. He also demonstrated that microfilariae ingested with the blood into the stomach of the mosquito tend to stay in the cardiac portion of the midgut immediately behind the proventriculus. They then travel forwards until they reach the proventriculus itself and escape into the perivisceral cavity of the thorax. Those which have moved to the pyloric region do not appear to have much chance of entering the haemocoel. On escaping into the perivisceral cavity from the proventriculus, the larvae enter the muscle bundles, either the vertical or the longitudinal ones. They move about in the spaces between the muscle bundles for some time before finally penetrating them. Inside the muscle they stretch out parallel to the muscle strands and then become dormant. They are always found in different stages of development in the thoracic muscles.

As the worms get older, their activity increases. During the last stages of metamorphosis the filariae, which up to this time have been at rest in the thoracic muscles, migrate and find their way to the connective tissues of either the thorax, legs, head or the proboscis.



Fig. 64

Development of *Wuchereria bancrofti* in *Culex fatigans*. A. After 3 days ; B. After 10 days.

During the course of development of larvae of *W. bancrofti* in the mosquito, chitinous encapsulation may take place. The encapsulation may be complete or partial. When partial, the filarial larvae are able to break loose from their chitinous sheath (Hu, 1938).

It was once thought that the larvae escape by rupturing a membrane at the apex of the labium on its dorsal side at the junction of the labium and the labella; this membrane has been called Dutton's membrane. The conjectures of Yamada and Komori (1926) that the filariae escape through the extreme tip of the labella have been confirmed by Rao and Iyengar (1932). Fully developed larvae of filaria are visible to the naked eye and when set free from the proboscis of the mosquito, they show very active movements.

According to Basu and Rao (1939) both temperature and relative humidity play a very important part in the transmission of *W. bancrofti* in man by *C. fatigans*. The optimum conditions for transmission found by them are 80°F and 90 per cent relative humidity. The same authors found a minimum of 12 microfilariae per 0.2 c.c. blood to be infective to mosquitoes, the highest infection being observed when the microfilaria count was 101 to 150 per 0.2 c.c. of blood.

Large numbers of microfilariae are generally fatal to the mosquito (Cruickshank and Wright, 1914). It is, however, a well recognised fact that on an average, mosquitoes take up a relatively greater concentration of microfilariae about 40 to 50 times more than is to be found in finger prick blood (Ashburn and Craig, 1907).

Although the population of *C. fatigans* is at its lowest during the monsoon, this is the most favourable season for the transmission of filaria by this species, the development of the worm during this time taking place within 10 to 12 days, while during the winter the embryos take 18 to 20 days to develop (Rao and Iyengar, 1932). Rao (1927), on the other hand, had previously found the highest natural infection during the winter months. These observations were made both in the laboratory and in the field in a highly endemic locality in Orissa (India).

In addition to *C. fatigans* the parasites may undergo a partial or complete development in many other species of mosquitoes. Rao and Iyengar (1932) tested the susceptibility of the common Indian species in this respect under experimental conditions and found complete development taking place in *Anopheles philippinensis*, *A. pallidus*, *A. annularis*, *A. stephensi*, and *A. sundicus*. According to them *Armigeres obturbans* and *Aedes vittatus* are totally refractory.

During artificial infectivity experiments with *A. stephensi*, it is a common feature to find filaria undergoing complete development in the body of this mosquito.

In China *C. pipiens* as well as *C. fatigans* and in Fiji and Polynesia *Aedes scutellaris* can act as efficient transmitters of *Wuchereria bancrofti*.

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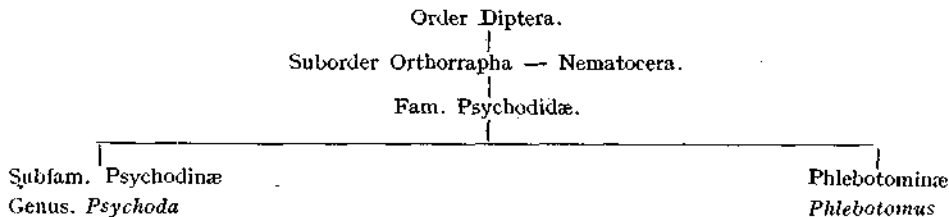
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Family PSYCHODIDAE (Sandflies)

Classification :—



The insects of this family are widely distributed. In size they are small or minute ; the body and wings are thickly covered with hairs. The antennae are long and filamentous and carry whorls of hairs at the joints. Metamorphosis is complete. The larva is a hairy maggot which lives on solids. Some larvae are aquatic and others live in damp, dark places. The adult is a fluid feeder. All are characterised by the 2nd long vein branching twice.

The Psychodidae are grouped into two subfamilies, namely, (1) Psychodinae and (2) Phlebotominae. They can be easily distinguished by the following characters: (1) Pose: While at rest the wings in Psychodinae are held roof-like over the abdomen, whereas in Phlebotominae they are held up. (2) Wings: The wings in the former are much broader and have the 2nd longitudinal vein branching for the first time very close to the root of the wings, whereas in the latter the wings are lance-shaped, i.e., narrow and pointed, and the first branching of the 2nd longitudinal vein takes place in the middle of the wings. (3) The external genitalia in Psychodinae consist of two pairs of claspers while three pairs are present in Phlebotominae.

Subfamily PSYCHODINAE:

A large number of species exist but none has yet been reported with certainty to suck blood.

These midges are popularly known as "moth flies" because of the peculiar roof-like position of the wings when at rest which gives them the appearance of tiny moths. This group of insects, also known as "owl midges", includes the commonest species of flies that breed in the bacterial films of filter beds used for the purification of sewage. They breed also in drains filled with decaying vegetable and organic matter.

As a rule they are harmless but Headlee and Backwith (1918) have drawn attention to two species, *Psychoda alternata* Say. and *Ps. phalacroides* Linn., of which large numbers may invade dwelling houses and contaminate food and water.

Both Patton and Evans (1929), and Okada (1927) have reported cases of intestinal myiasis caused by larvae of *Ps. allipennis* and *Ps. punctata* respectively.

Psychoda midges have a very short life cycle and on account of its great productivity these flies have been suggested to be ideally suitable for employment in genetical researches.

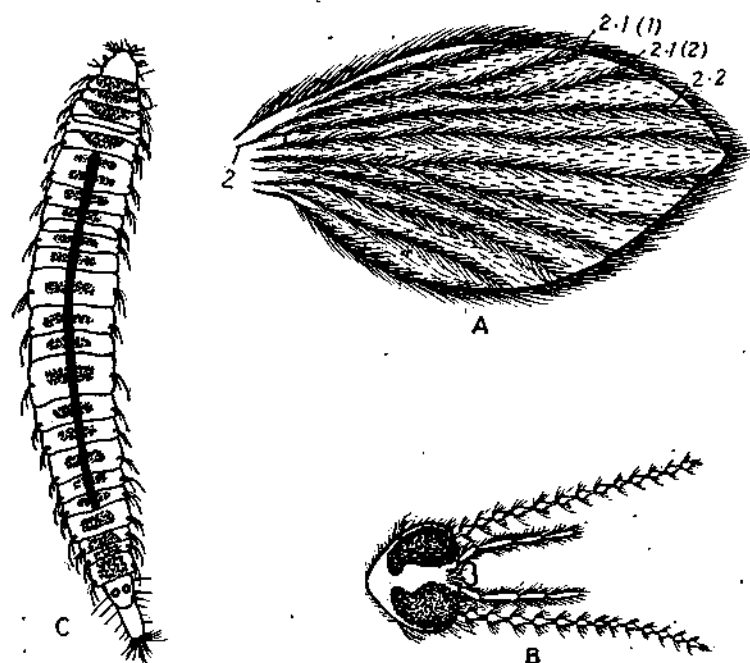


Fig. 65
Psychoda.
A. Wing; B. Head; C. Larva.

Its breeding can be checked by means of bleaching powder.

Subfamily PHLEBOTOMINAE.

This contains only one genus *Phlebotomus*. They are important as the females are blood-suckers and some of them are proved carriers of certain disease organisms.

Genus *Phlebotomus*. They are small and are of a greyish-brown colour. They are extremely delicate. They have a wide distribution in the moderately warmer regions of the world. All sand-flies are incapable of flying any long distance like a mosquito; they move by hopping from one place to another and by this character alone they can be readily recognised in nature.

Head:

Antennae are long and slender and are composed of 16 segments. They carry whorls of hair at the joints and are similar in the two sexes. The palpi are long and flexible, and consist of 5 segments which are clothed with scales and hairs. The proboscis is composed of (1) the labium which conceals the true cutting and piercing organs, (2) a pair of mandibles which are broad and serrated at the edges, (3) a pair of maxillae which are much narrower than the mandibles, and bear a

number of teeth on one edge, (4) the labrum—epipharynx, and the hypopharynx, the tip of the latter being pointed and the edges near the tip set

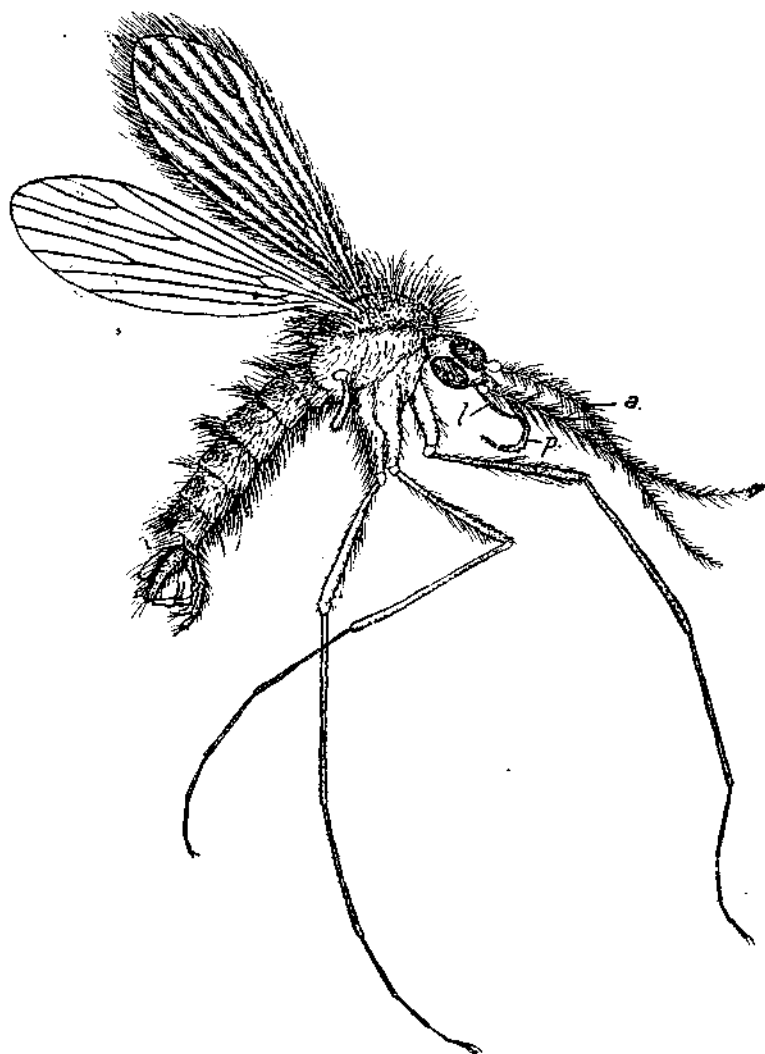


Fig. 66
Phlebotomus argentipes, male.
a, antenna; p, maxillary palp; l, labium.

Thorax:

The thorax is mainly composed of mesothorax. The legs are much longer in proportion to the size of the insect and slender.

The wing is densely hairy. The second longitudinal vein branches twice, the first time in the middle of the wing. Scales are entirely absent.

Abdomen:

The abdomen which is clothed with hairs consists of 10 segments, the last two being adapted for sexual purposes. The sexes are differentiated by the abdomen

which is narrow and is often slightly upturned posteriorly in males which also possess claspers. These are attached to the last abdominal segment. In females

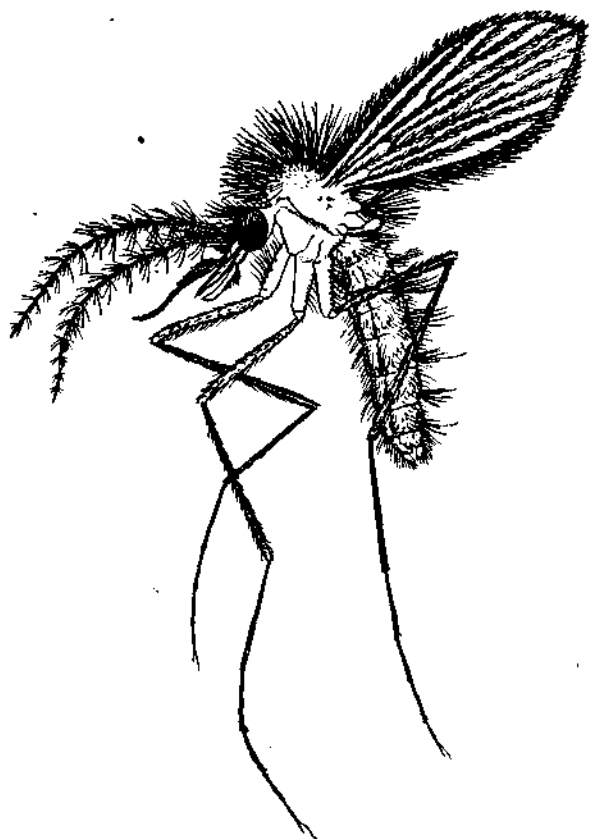


Fig. 67
Phlebotomus argentipes, female.

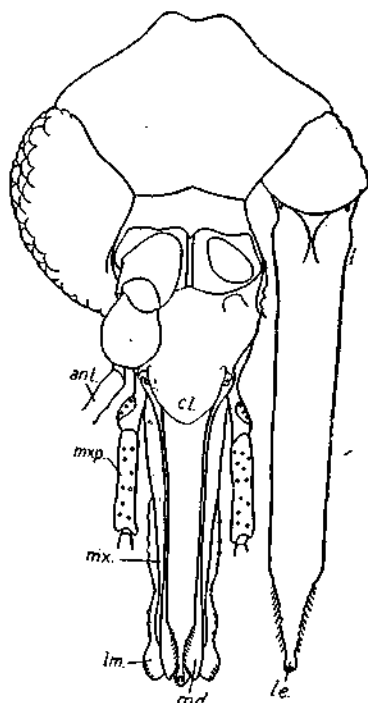


Fig. 69
Mouth parts of *Phlebotomus*. -
ant, antenna; cl, clypeus; le, labrum-epipharynx; l, labium; mx, maxilla; mxp, maxillary palp; (After Christophers, Shortt and Barraud).

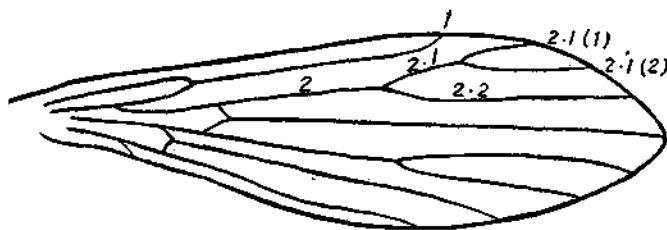


Fig. 68
Wing venation of *Phlebotomus*.

the abdomen is round and claspers are absent. The male terminalia are arranged in five pairs; (a) superior, (b) inferior, (c) submedian lamellae, (d) intromittent organ, and (e) intermediate appendages. The superior clasper is provided at the distal end with spines; their position and their character are helpful in the identification.

Alimentary canal.

(1) Buccal cavity which lies at the base of the clypeus; (2) Pharynx; (3) Oesophagus with a rounded diverticulum; (4) Midgut; (5) Hindgut; (6) Two pairs of Malpighian tubules; and (7) Rectum.

The salivary glands consist of two lobes. The ducts unite near the mid-region of the head forming a common duct which enters the buccal cavity.

The buccal cavity in sandflies consists of a ventral plate, and two dorsal ones which unite posteriorly to form a single thick pigmented plate. The ventral plate bears at its posterior margin two systems of teeth which project horizontally into the lumen. The armature of the pharynx consists of ridges and blunt teeth in the

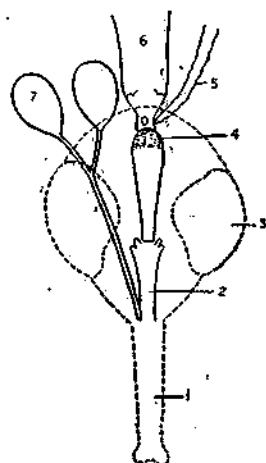


Fig. 70

Anterior part of the alimentary canal of *Phlebotomus argentipes*.

1. prosboscis; 2. buccal cavity; 3. eye; 4. posterior part of pharynx; 5. duct of diverticulum; 6. midgut; 7. salivary glands.

(After Shortt, Barraud and Craighead.)

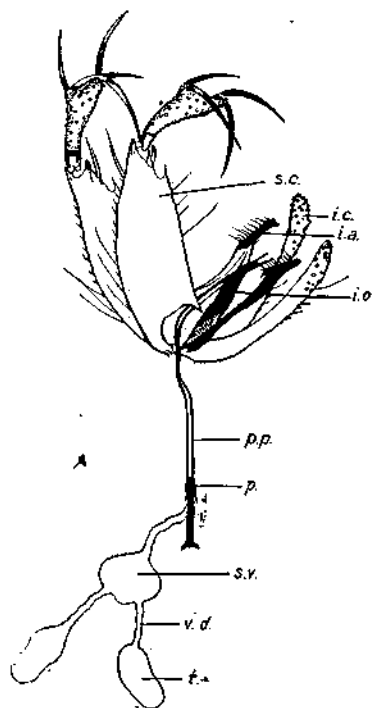


Fig. 71

Generative organs and terminalia of male *Phlebotomus argentipes*.

i.a., intermediate appendage; i.c., inferior clasper; i.o., intromittent organ; p., pompetta; p.p., paired penis; s.c., superior clasper; s.v., seminal vesicle; v.d., vas deferens; t., testes.

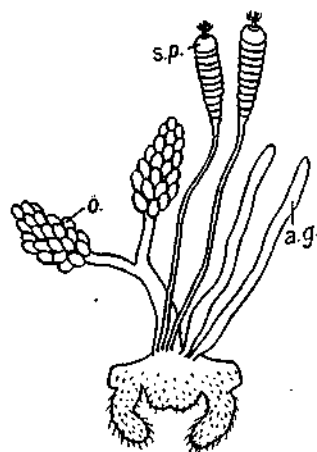


Fig. 72

Internal generative organs of a female *Phlebotomus argentipes*. a.g.,—accessory gland. o.,—ovary.

s.p.,—spermathecae or seminale vasculæ

posterior part. In the male the armature of the pharynx and the buccal cavity is much less developed.

Reproductive system.

Male: (1) Testes ; (2) Seminal vesicle ; (3) Ejaculatory duct connected with a pump (pompette) which regulates the flow of spermatozoa ; (4) The ejaculatory duct is then continued with the penis or the intromittent organ which consists of a pair of long slender and highly chitinised organs ; these lie between the intermediate appendages.

Female: (1) Ovary ; (2) Spermathecae which differ widely in various species ; their structure is of specific importance. The spermathecae lie in the median line in the region of the oviduct. They consist of a single thin-walled sac and are relatively very large. At their junction with the oviduct they are strongly chitinised and are transversely ridged on their inner surfaces.

LIFE HISTORY AND BIONOMICS.

The egg is comparatively large and torpedo shaped. It possesses some longitudinal wavy lines on the outside. Any damp moist place, where there is sufficient

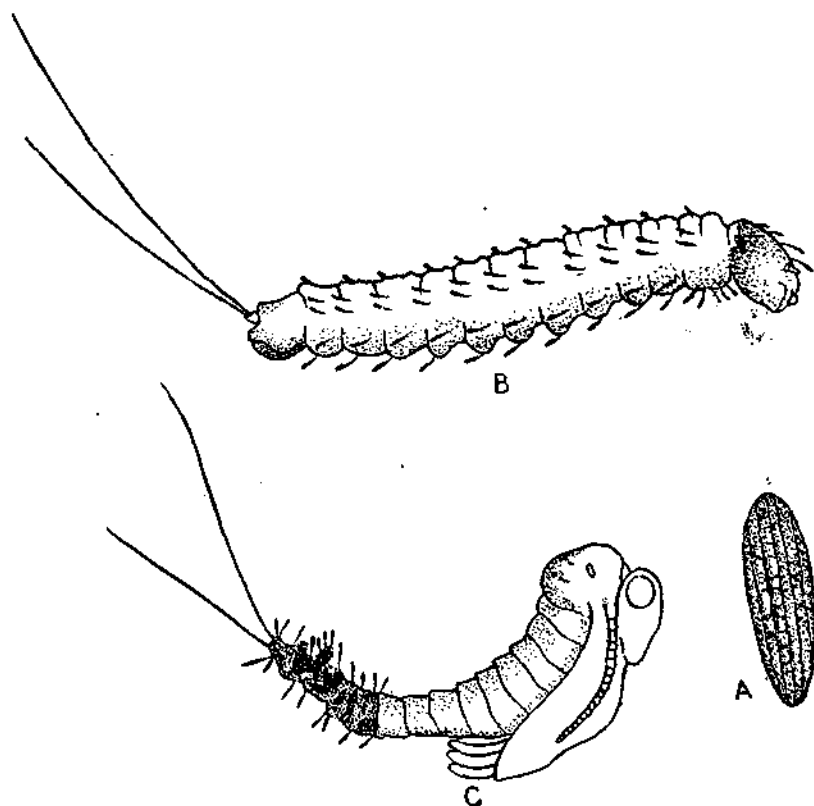


Fig. 73
Showing complete metamorphosis of a sandfly.
A, egg; B, larva ; C, pupa.

moisture, absence of light, and where organic matters especially excrement of animals are present in sufficient amount, is selected by the female for oviposition. The

egg, on account of its small size, is never noticed in nature but sites of oviposition can be guessed by finding larvae. In the laboratory the egg stage lasts in the case of *P. argentipes* from 3-4 days during the rainy season and is extended to 7 days in winter.

The larva is a foot-less hairy maggot. It has a distinct head, 3 thoracic and 9 abdominal segments. The last segment carries two pairs of long bristles which are kept erect; among them one pair is remarkably long. The mouth is provided with mandibles; eyes are absent. The body is covered with spines in more or less regular transverse rows. This stage lasts from 2 weeks to a longer period depending on the temperature.

The pupa has always the last larval skin attached to it. The abdomen of the pupa is curved upwards. The integument is clothed with minute spines. The minimum life of the larval and pupal stages combined is about 30 days in Calcutta during the rainy season.

The blood-feeding habit of the adult is restricted to the female alone; the males live entirely on vegetable sap.

The presence of the sand-fly is scarcely observed. It is the bite which is first felt. The bite is irritating and the sensation is unlike that experienced in the case of mosquitoes.

Sand-flies are particularly annoying to man at night when there is little or no breeze and the atmospheric temperature is high. They are nocturnal in habits and only a few are attracted to light. They usually attack the lower extremity rather than the upper and the back in preference to the front part. The face is seldom attacked. They can bite through thin clothing.

All sand-flies are exceptionally small and it is only the finest net that will prevent them from entering through the meshes.

Natural enemies are lizards and spiders.

P. argentipes. This midge is so named on account of its possessing silvery white feet. By this character alone it can be readily recognised in the fresh state.

Smith, Mukerjee and Lal (1936) reported that larvae of *P. argentipes* are generally found in rural areas in soil collected within a radius of 20 yards of a dwelling house or cattle shed. In cattle sheds a favourite breeding site is under the earthen-ware feeding troughs. The larvae penetrate to a depth of 3 or 4 inches in loose and sodden soil. Rat burrows do not yield any larva though in urban areas these often are the favourite breeding sites. Cattle sheds harbour a much larger number of larvae and adult flies than do human dwelling houses. In fact larvae are found in places where the requisite conditions for breeding are fulfilled and the food of the adult is near at hand. It is for this reason that larvae are particularly found in cattle sheds and poultry houses.

The larvae after hatching migrate over a wide area of the soil protected from the sun and the rain, and penetrate some inches below the surface in suitable situations. The presence of larvae in the soil is detected by the flotation method of Young, Richmond and Brendish (1926) and for which sugar solution is used.

Under the most favourable conditions the whole life cycle takes about 30 days, the egg, larval and pupal stages requiring 4, 14, and 7 days respectively. In winter

it takes 2 to 3 months. If fed on the 2nd day, they oviposit on the 5th or 6th day and may die immediately afterwards. If not mated, the female will take another meal on the 5th day and survive until the 9th or 10th day; occasionally they may be induced to feed a third time when they will survive until about the fifteenth day (Napier and Smith, 1926).

Sand-flies do not feed again till they have completely digested the previous blood meal and oviposited; this process takes approximately 3 days in mated flies and much longer in unfertilised ones. About 30 per cent of flies will survive until the 3rd feed about 6 to 7 days after emergence.

For the transmission of *L. donovani* by the bite of the sand-fly, it is necessary that the sand-fly should live for at least 6 days after an infecting blood meal. By the 6th day or by the time the sand-fly is ready for its third feed (since these midges feed every 3rd day) flagellates are usually found to have invaded the pharyngeal and buccal cavities. To determine whether sand-flies live at least for this period Smith, Mukerjee, Halder and Lal (1936) released flies of known ages marked by fluorescent powder. Only three or four per cent of them were recaptured on the 6th day and only a few up to the 15th day after release.

Smith, Halder and Ahmed (1940) found that about half of the sand-flies that take a first blood meal and then feed on raisins are available at the end of 10 days for feeding.

There is generally a heavy mortality of sand-flies on the 4th or 5th day after experimental feeding due to difficulties in oviposition. This can be prevented by placing the insects at an uniform temperature of 28°C. Under such conditions oviposition takes place on the 3rd or 4th day after feeding (Shortt, Barraud and Craighead, 1926).

P. argentipes is known to feed in nature on man, cattle and goats, but it shows a decided preference for cattle (Lloyd, Napier & Smith, 1925).

Lloyd and Napier (1930) later compared the feeding habits of different sand-flies of Bengal and they found that *P. argentipes* and *P. papatasi* feed almost exclusively on the blood of cattle or man. *P. minutus* also usually feeds on the blood of cattle or man, but also quite frequently on the blood of other species. *P. minutus* is a more persistent human-blood feeder than *P. argentipes*, and *P. papatasi* feeds on human blood less readily than the other two species.

The percentage of flies containing human blood appears to vary in inverse ratio to the distance of human sleeping quarters from the cattle sheds where the flies were caught.

P. argentipes are found more in cattle sheds and chicken houses than in human sleeping quarters. These flies are seldom found in any of the upper rooms. In the cattle sheds they are even found in the day time in dark corners. This species is rarely attracted to artificial light.

Natural infection: A gregarine, *Monocystis mackiei*, has been reported in the intestine of *P. argentipes* (Shortt and Swaminath, 1927).

P. papatasi. While *P. argentipes* are more numerous in cattle sheds, *P. papatasi* are caught in large numbers in human dwellings.

It is also a notorious biter of man. It is far more active in its habits than *P. argentipes*. According to Anderson (1939) *papatasii* is apparently capable of flying as high as 70 ft. above the ground and he found no evidence that persons sleeping in upper storeys were in any way less exposed to attack. In Malta, Newstead (1911) found them more frequently on the first floor than in rooms at a greater elevation. On water alone *papatasii* can be kept alive for 6 days. Adler and Theodor (1929) caught *papatasii* in large numbers in fowl houses with signs of the flies having fed on fowls' blood; he also found them feeding on geckoes in captivity while Knowles (1929) thought that it does not feed on lizards at all.

Whittingham and Rook (1922) have worked out its life cycle and they found the whole cycle occupying from 7 to 8 weeks during August.

Adler and Theodor (1925) found oöcysts of *Hepatozoon* in this species.

P. minutus. This species does not readily feed on man but feeds chiefly on cold-blooded animals, especially lizards (Howlett, 1913). It is widely distributed in this country.

P. sergenti. Evidence has been collected by Sergeant brothers etc. (1921) and later by other observers suggesting that this species bites man in nature.

Common sand-flies found in Calcutta.

P. argentipes Ann. and Brun.

P. papatasii Scop.

P. minutus Rond.

P. squamipleuris Newst.

SAND-FLIES AND DISEASES

<i>P. argentipes</i> Ann. and Brun.	...	Kala azar in India.
<i>P. chinensis</i> Newst.	...	Kala azar in China.
<i>P. sergenti</i> Parr. and <i>P. papatasii</i> Scop.	...	Oriental Sore.
<i>P. papatasii</i>	...	Sand-fly fever.
<i>P. chinensis</i> and <i>P. sergenti</i> var <i>mongolensis</i>	...	Canine leishmaniasis in China.
<i>P. perniciosus</i> Newst. (Algeria, Malta, Marseilles etc.)	...	Canine leishmaniasis.
<i>P. major</i> Ann. (Greece)	...	"
Varieties of <i>P. major</i> . (in other places)	...	"

Kala-azar. The flagellated stage of *Leishmania donovani* can be demonstrated in the anterior part of the midgut of *P. argentipes* when they are dissected on the 5th day after feeding on a case of kala-azar. (Knowles, Napier and Smith, 1924). Free forms are also seen as far forward as the middle of the pharynx. Recently Swaminath, Shortt and Anderson (1944) have succeeded in transmitting the disease to volunteers by the bites of *P. argentipes*. These sand-flies after feeding on the blood of K. A. patients were maintained at 28°C on raisins until they were fed on the volunteers. The distribution of kala-azar also coincides with that of *P. argentipes*.

In China Patton and Hindle (1927) have proved *P. chinensis* a vector of kala-azar in that region. Sun and his collaborators (1936) found *P. chinensis*

naturally infected with flagellates morphologically indistinguishable from those of *Leishmania donovani*. These flagellates were proved to be those of *L. donovani* by later experiments reported by Yao and Wu, (1941).

Oriental sore. The epidemiological in addition to experimental evidence points to the fact that *P. papatasii* and *P. sergenti* can both act as carriers of Oriental sore.

Sergeant brothers and their collaborators (1921) found *P. papatasii* responsible for the spread of Oriental sore in Algeria. Their findings have later been confirmed by Adler and Theodor (1925, 1926) and by Adler and Ber (1941). Sinton (1922, 1924, 1925,) in a careful study of the distribution of Oriental sore in India was able to show a close correlation between it and that of *P. sergenti*, and later Shortt, Sinton and Swaminath (1936) discovered a definite host parasite relationship between *P. sergenti* and the Punjab strain of *L. tropica* which readily develops in this species. They produced Oriental sore in a monkey with flagellates from *P. sergenti* 5 days after it was infected. It may be noted that Adler and Theodor (1929) experimenting with both *P. papatasii* and *P. sergenti* in regard to the development of Baghdad strain of *tropica*, found *papatasii* a better insect host than *sergenti*. They have also found *papatasii* infected with *L. tropica* in nature (Adler and Theodor, 1929).

Therefore all indications point to the transmission in nature taking place by the bite of the sand-fly, the mechanism being similar to that observed in the case of the parasites of visceral leishmaniasis developing in *P. argentipes*.

Sand-fly fever. Both Taussig (1905) and McCarrison (1906) drew attention to a special type of short fever which they had suspected of being carried by sand-flies, hence the name sand-fly fever was given to it. Through the researches of Doerr (1908) and Doerr and Russ (1909) it became known that the blood is infective during the first two days of the illness and *P. papatasii* is the carrier of this disease. From Whittingham's observations it became known that recently bred adult is capable of infecting human beings and that the midge is more than a direct vector. Apparently the virus does not attain pathogenicity or is not transmitted until the fly has lived for some days and has already had and digested a feed of blood. Infected sandflies brought to England from Malta oviposited before death and the resultant adults were found capable of infecting human beings. The adult is able to suck blood within 24 hours after emergence.

Whether such natural conveyance of infection from adult fly to its brood, or the alternative, that the larva acquires the virus by feeding on the infected faeces passed by the parent or by consuming the dead body of the parent, is the true explanation has not yet been determined.

The virus of sandfly fever is now regarded capable of passing to the next generation through the ovum. There is no evidence yet that any other insect or other species of sandfly can transmit the disease.

Leishmania infantum and *canine leishmaniasis*. These diseases which are common in the Mediterranean littoral were at one time thought to be carried by fleas (Basile, 1911). It is now known or it has been assumed that *P. perniciosus* is the vector of this type of *Leishmania* in Algeria, Malta and Marseilles, *P. major* in Greece and *P. sergenti* and varieties of *P. major* in other places. In certain parts

of North Africa the vector is probably *P. papatasi* as the local strain of *Leishmania* has been found to multiply better in this species than in *P. sergenti*. Adler and Theodor (1930) found *P. perniciosus* infected with *Leishmania infantum* in nature.

In China where canine leishmaniasis is quite common *P. chinensis* and *P. sergenti* var. *mongolensis* are the principal carriers of this disease. (Feng and Chung, 1939; Chung and Feng, 1939).

Oroya fever and Verruga. Townsend (1915) has taken it as proved that a species of *Phlebotomus* is the transmitter of both oroya fever and verruga which exist endemically in Peru, and that these are stages of the same disease. It has been postulated by the author that the organisms are introduced into the blood by *Phlebotomus*. Noguchi (1926) has shown that *Bartonella bacilliformis* can be transmitted from infected to healthy monkeys by bites of *Dermacentor andersoni*. There is, however, some indication that Oroya fever is transmitted to man through the agency of some blood-sucking arthropod and except for the experimental transmission of the disease to monkeys by *D. andersoni*, this theory has not been established.

Espundia. This disease which is peculiar to certain parts of South America and the Anglo-Egyptian Sudan is a condition of muco-cutaneous leishmaniasis in which the exact mechanism of infection is not known. No light has hitherto been thrown on the part insects, particularly sandflies, play in the propagation of this disease.

Breeding of Sand-flies. Waterston's method of breeding sand-flies, as modified by Smith (1925) in the case of *P. argentipes*, is given below.

Blood-fed females captured in nature should be kept in glass tubes for two or three days during which time all the blood in the midgut will be digested. The flies are then to be transferred to another tube set on a Plaster of Paris cell, in which are placed a few small stones and some crushed rabbit's faeces. The glass tube in which the flies are enclosed should be slightly larger than the hollow in the plaster cell. The cell is then placed on a rough earthen-ware vessel with a layer of damp cotton-wool between the plaster and the earthen-ware vessel. The whole apparatus is then placed on a Petri dish containing water. This will keep the earthen-ware vessel and the plaster of Paris cell in just the requisite amount of moisture to favour oviposition. Eggs laid are found on the sides of the hollow in the plaster cell. In this position they should be left till the

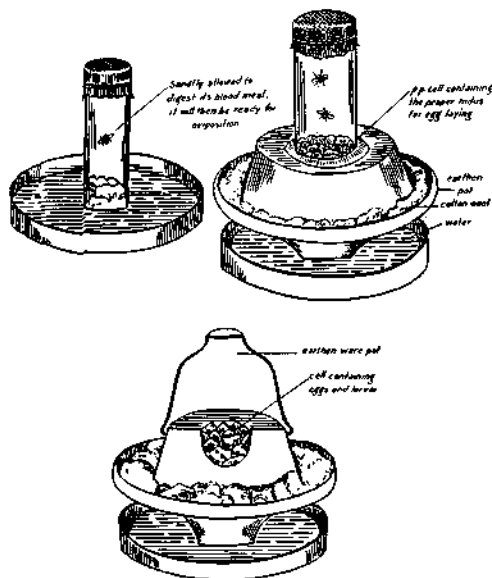


Fig. 74
Breeding sandflies in the laboratory. (After Smith).

larvae emerge and make their way to the rabbit's faeces to feed. The larvae generally emerge in 4 to 7 days and thereafter the glass tube from the top of the tray should be removed and a second earthen ware vessel used to cover the plaster cell, for the larvae grow and thrive better in the dark. As the larvae grow, they require less and less moisture, till during pupation practically no water is required. Pupation takes place in 12 to 14 days and the stage of pupation usually lasts 5 to 10 days. As soon as pupation takes place, the upper earthen-ware vessel is removed and the chimney of a hurricane lamp is substituted. A piece of thin muslin is used to cover one end and the other is fixed to the plaster tray by plasticine.

Collection.

(1) They can be collected in the morning or evening in dark and damp rooms with the help of a torch. They are captured in a test-tube by inverting it over the insect.



Fig. 75

An aspirator for catching small insects especially midges. (After Buxton).

(2) Buxton (1928) recommends the use of an aspirator which consists of a glass barrel 3 inches by 1 inch, a nozzle of glass tubing and an outgoing piece of tubing, protected by gauze and connected by rubber tubing to a mouth-piece. It can be used for catching any small insect. The insects are sucked into the barrel. Kirk and Lewis (1940) also found an aspirator very useful in capturing sandflies.

(3) Traps: Traps have often been used to obtain sand-flies from the burrows of animals. These have been devised by Vlasov (1931) and Petrischeva (1935) in the U.S.S.R. and consist of strips or flags of paper, smeared with castor oil and held in cleft sticks by means of which they can be fixed across the mouth of the burrow. Sand-flies entering or leaving the burrow and accidentally touching the paper are immediately caught on the paper.

Dissection.

(1) The method of dissection of the alimentary system is the same as in mosquitoes. After the skin on both sides between the last and the penultimate segments has been cut, the abdomen is pulled and thereafter the head is severed from the neck. By pulling the last segment, the whole alimentary canal may be exposed.

(2) After the insect has been treated with spirit and then salt solution, the two terminal segments are removed. Mukerji (1930) advises that the border of the head behind the eyes is to be gently teased with a lancet-shaped needle with a motion that will tend to separate the head from the rest of the body. A careful and steady teasing will separate the head showing clearly the proventriculus, the paired globular salivary glands at the distal margin of the head, as well as a portion

of the oesophageal diverticulum running parallel to the proventriculus. At this stage the excess fluid is to be soaked up. Successive short and steady pulls, directed towards the abdominal end, by holding the lateral edge of the thorax will allow the emergence of the entire alimentary canal.

(3) After the avulsion of the legs and wings the fly is treated with rectified spirit for a few seconds and then shaken in saline solution. Dissection is performed in saline solution on a glass slide under a dissecting microscope with the help of two needles. The head is carefully separated from the thorax by placing one needle across the base of the head and the other across the front of the thorax exerting traction of the head in a forward direction. If carefully done the anterior portion of the alimentary tract will be drawn out, still attached to the head and unbroken midgut.

The terminal segments of the abdomen are now dissected by nicking the chitin with a needle on either side of the abdomen near the genitalia and gently drawing out the hindgut and the spermathecae. The hindgut is cut through close to the anus and the terminal segments of the abdomen with the attached spermathecae are placed in a drop of lactophenol (carbolic acid crystal one part, glycerine 2 parts, lactic acid 2 parts and distilled water 1 part). The remainder of the alimentary tract is drawn out through the thorax by exerting traction on the head in a forward direction. It may be necessary to remove the head capsule in order to expose the buccal cavity and the pharynx. The rest of the alimentary canal has already been exposed. (Sinton, 1932).

IDENTIFICATION.

For identification alone the fresh insect is placed in a drop of lacto-phenol and the head and terminal segments of the abdomen are separated from the body. The head is arranged ventral side uppermost, a cover-glass applied and slight pressure exerted. The terminal segments of the abdomen in the female are treated in the same way to demonstrate the form of the spermathecae, which in case of difficulty can be found by following the spermathecal duct.

The dry specimen should first be wetted with alcohol and then treated with caustic solution before it is prepared for identification as stated above.

Male. Males are identified chiefly by the character of the spines on the superior claspers.

Female.

The Indian species of *Phlebotomus* may be placed in 3 main divisions, (1) The erect-haired division, (2) the recumbent-haired division and (3) the intermediate group.

Erect-haired group: The members of this division have always some erect hairs which are numerous on the dorsal aspect of segments II to VI of the abdomen.

The heavily chitinated parts of the bases of the spermathecae of the females of this division are segmented in their entire lengths, buccal armature and pigmented area absent or very rudimentary. This group includes all *Phlebotomus* which are likely to be associated with the transmission of disease to man in India.

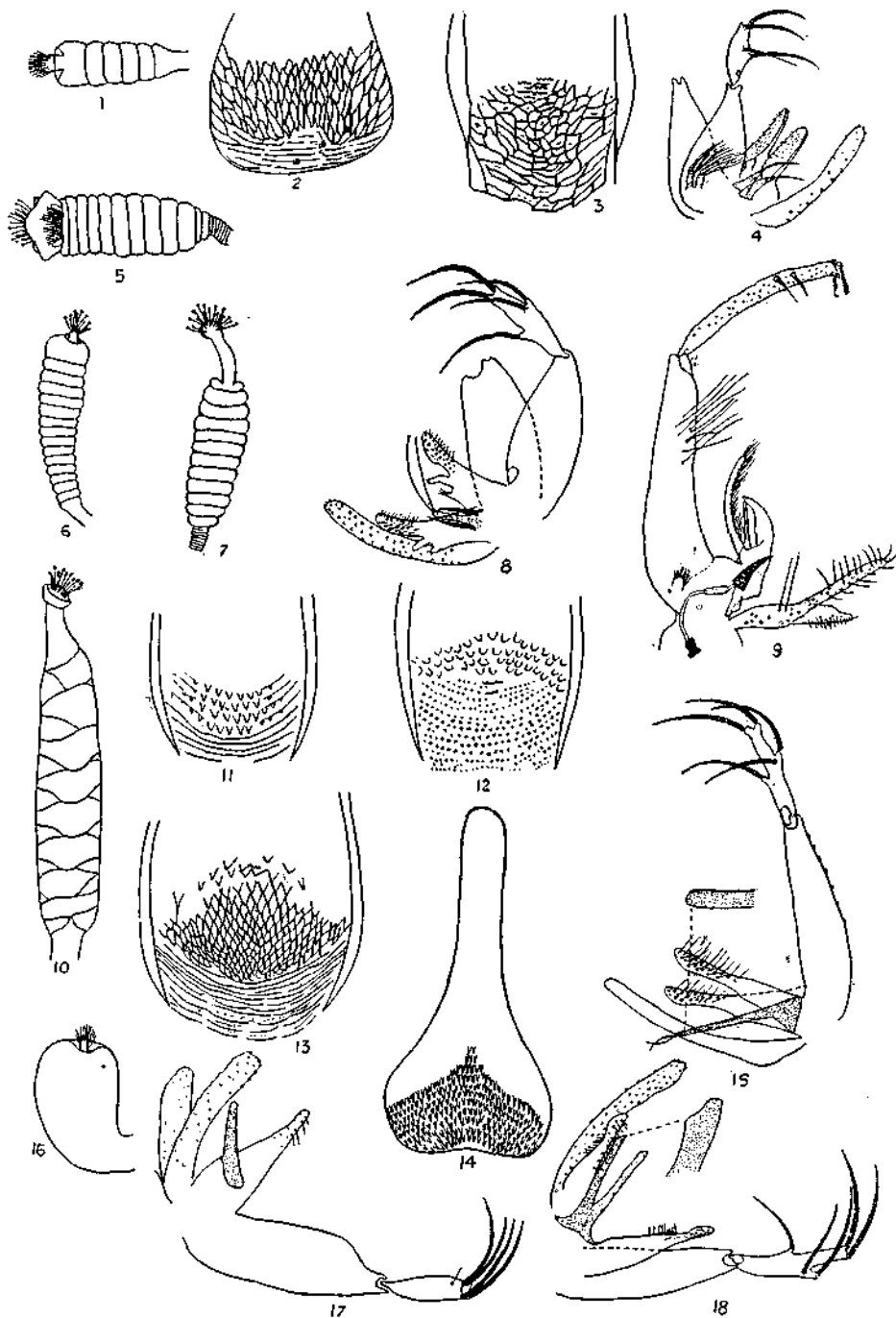


Fig. 76

Spermatheca, pharyngeal armature and male hypopygium of some important disease-carrying *Phlebotomus*. (After Sinton).

1. Spermatheca of *P. sergenti*. 2. Pharynx of female *P. sergenti*. 3. Pharynx of female *P. papatasi*. 4. Hypopygium of *P. sergenti*. 5. Spermatheca of *P. papatasi*. 6. Spermatheca of *P. argentipes*. 7. Spermatheca of *P. major*. 8. Hypopygium of *P. argentipes*. 9. Hypopygium of *P. papatasi*. 10. Spermatheca of *P. chinensis*. 11. Pharynx of female *P. argentipes*. 12. Pharynx of female *P. major*. 13. Pharynx of female *P. chinensis*. 14. Pharynx of female *P. minutus*. 15. Hypopygium of *P. major*. 16. Spermatheca of *P. chinensis*. 17. Hypopygium of *P. chinensis*. 18. Hypopygium of *P. chinensis* (detail).

Recumbent-haired group: In this division the dorsal abdominal hairs on segments 2—6 are all recumbent ; the body of the spermathecal chitinizations usually with a smooth outline and any traces of segmentation if present are confined to the distal end. The buccal armature and pigmented area are usually well developed and have a specific morphology. This division includes *P. minutus*, *P. montanus* etc.

Intermediate group: *P. squamipleuris* is contained in this group.

The identification of the five important species of sand-flies can be easily effected with the help of the diagrams. For other species the diagnostic tables constructed by Sinton (1932, 1933) for both males and females will be of invaluable assistance. Prophylactic Measures Against Phlebotomus.

- (a) Abolition of breeding grounds.
- (b) Cracks, crevices and holes must be closed for which tar may be used, if necessary.
- (c) Destruction of the adult fly by spraying with cresol, D.D.T., and pyrethrum.
- (d) Fumigation: The method is too expensive and too laborious.
- (e) Repellents: Paraffin, camphor and citronella oil are efficacious.
- (f) Cotton net of at least 45 meshes to the square inch is recommended.

Measures Against Kala-Azar.

Preliminary field experiments carried out by Wu, Ghosh, McClymont and Roy in a village near Calcutta indicate the great possibilities of controlling kala-azar by destroying sand-flies by spraying with a kerosene emulsion of D.D.T. or pyrethrum. D.D.T. was found to be definitely more efficient in this respect. The floor and the lower part of the walls of cattle sheds abounding in sand-flies were treated with the insecticidal fluid once a fortnight. One application proved sufficient to stop the breeding of sand-flies for at least 8-10 days. It also came out in course of this work that sand-flies (*P. argentipes* and *P. minutus*) did not show any tendency to migrate from the untreated to the treated parts of a big cowshed.

Preservation: Pinning always injures flies to such an extent that identifications are rendered impossible. When preserved in spirit, the hairs are all lost ; they are particularly important for identification.

The insects should be placed on a web-like layer of teased cotton-wool contained in a shallow pill box or a corked tube but they must not be covered with wool as the appendages are thereby likely to be damaged.

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Family CHIRONOMIDAE

The insects belonging to this family are delicate midges which resemble mosquitoes very closely but can be easily distinguished by (1) the thorax, (2) wing venation, and (3) proboscis. The anterior part of the thorax extends over the head. The wing is devoid of scales and the venation is quite different from that of mosquitoes. The proboscis is much shorter.

The eggs are laid in a transparent jelly-like mucus in stagnant water. The larvae are aquatic. The larva of one species on account of the presence of red pigment in the haemolymph is popularly called blood worms. They are found in pools, ponds, ditches, drains etc. They are also found in settling-tanks of water-works and may at times by their numbers partly block some pipes. They generally live in tubes formed of mud.

The adults are harmless and all live on vegetable sap.

Family CERATOPOGONIDAE.

This family was formerly considered a sub-family of Chironomidae but has been raised to the status of family by Malloch in 1917. The difference between the two is shown below.

In Ceratopogonidae the mandibles are well developed and the thorax does not usually project over the head. In Chironomidae the mandibles are absent and the anterior part of the thorax arches forwards over the head.

Out of numerous genera of this family only four are blood suckers ; these are :

(a) *Culicoides*, (b) *Lashiohelea*, (c) *Leptoconops*, and (d) *Acanthoconops*. The females alone bite, males being vegetable feeders.

Genus *Culicoides*. Lat.

The wings are blotchy or dappled ; macrotrichia of wings distinct ; empodium very short.

It is almost world-wide in distribution. The larvae are generally aquatic or else are found in rotting vegetation.

Genus *Lashiohelea* Kieff. Wings are not dappled ; they have dense macrotrichia all over ; costa extends to about the middle of the wing ; empodium very long.

The larvae are terrestrial and breed in moist decomposing vegetable matter.

Genus *Leptoconops* Skuse. Costal vein does not extend beyond the middle of the wing ; the first and the third veins are fused ; presence of an extra vein (really

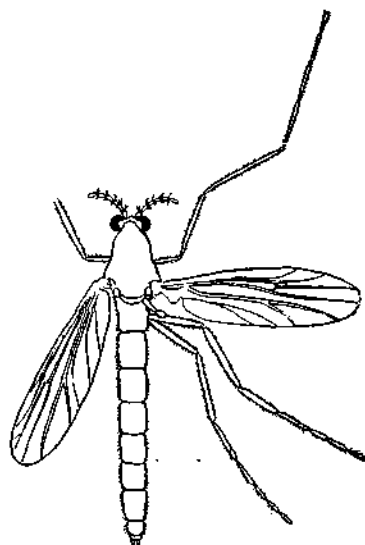


Fig. 77
Chironomus.

a fold of the wing) between the third and the fourth longitudinal veins. The antennae of the female consist of 13 segments.

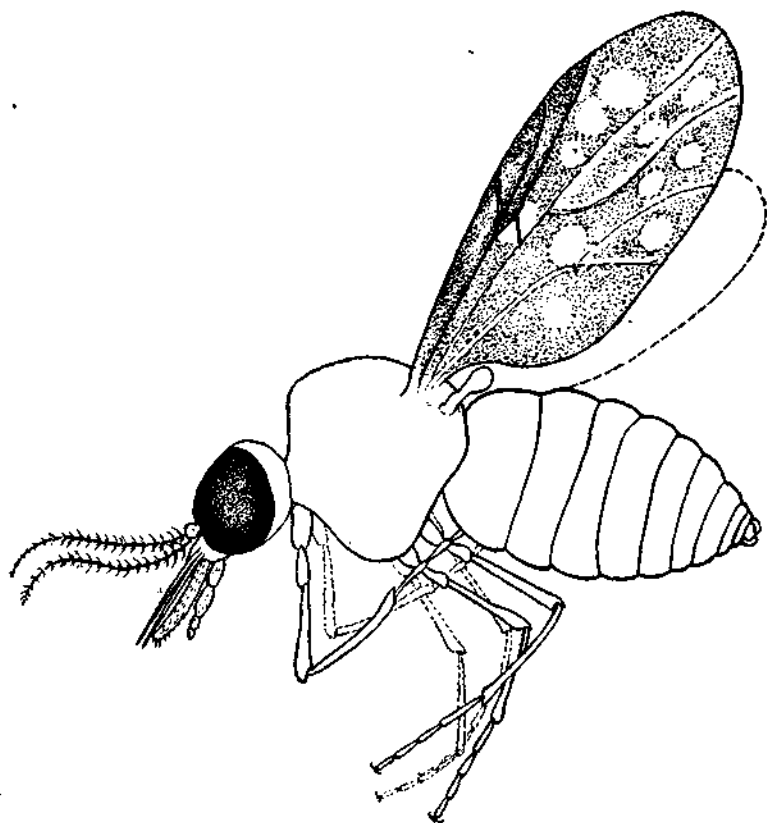


Fig. 78
Culicoides.

The females of several species are known to bite man. They sometimes attack domestic animals in swarms. The bites are painful and the subsequent local reaction is irritating and persistent.

The majority are found in the tropics.

Genus *Acanthoconops*. Carter. This is distinguished from *Leptoconops* in having the front clothed with bristles or spines.

Genus *Culicoides*. They are minute insects and are able to inflict painful bites and at times in certain localised areas they constitute a greater nuisance than mosquitoes. They generally occur out of doors and are seldom found inside the house. They bite mostly after sunset. In addition to cattle and other domestic animals, some bite man. Some even bite caterpillars, snails, earthworms, lizards etc.

The adult is a small, delicate midge. The sexes are differentiated by the antenna which is plumose in males and pilose in females. The eyes in the male are

more widely separated than in the female. The proboscis is longer in the female than in the male. The labium is fleshy. The labrum is armed with teeth on both its lateral edges near the tip. The mandibles and maxillae are each armed with several strong teeth. The hypopharynx is finely toothed at the tip.

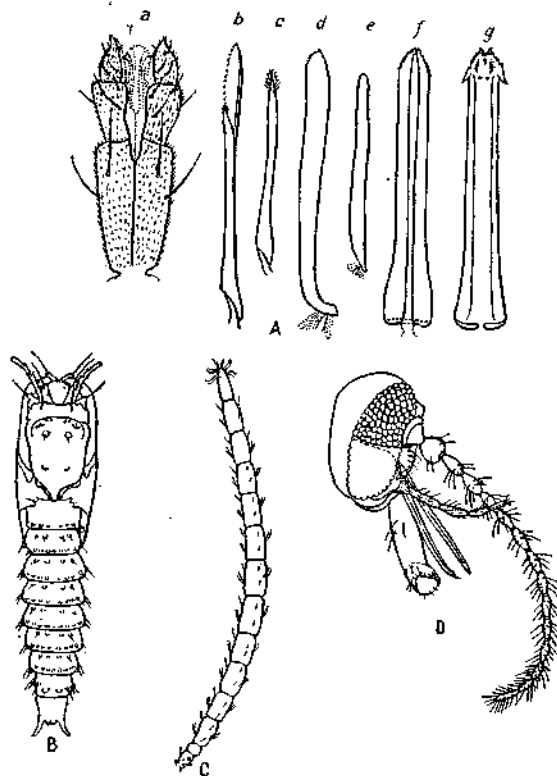


Fig. 79

Mouth parts of *Culicoides austeni*. (after Carter, Ingram and Macfie).

A. a,—labium, ventral view (female); b,—maxilla (female); c,—maxilla (male); d,—mandible (female); e,—mandible (male); f,—hypopharynx (female); g,—labrum (female);

B. Pupa;

C. Larva;

D. Head of female *Culicoides*;

The larvae are found in such places as pools, tree holes, rotting of banana plants etc. They are generally aquatic. The mature larva consists of the head, three thoracic and ten abdominal segments. The body is slender and is sparsely hairy. Its presence is readily detected by its rapid vibratile movements which closely simulate those of a spirochaete. The pupa has well developed spines at the sides. The pupa may either remain afloat on the surface of water or anchor itself to some object by two prominent terminal spines.

The duration of the different stages of *C. oxystoma* in confinement is as follows: egg stage lasts 3-11 days, larval stage 2-10 weeks, and the pupal stage 3-7 days (Patel, 1922).

Culicoides occurs throughout India particularly in Assam and Bengal, some species being found almost throughout the year.

They are identified mainly by the wing pattern and male hypopygium.

Among others the following species are common in this country. *C. oxystoma* Kieff: feeds on mammals and seems to prefer horses, cattle, buffalos and goats.

C. fulvithorax Aust. Feeds on large domestic animals also bites man. *C. anopheles* Edw.: normally it bites cattle but occasionally it will be found attached to the abdomen of female *Anopheles* mosquitoes and sand-flies.

Several species of *Culicoides*, especially *C. austeni* and *C. grahmi*, (Sharp, 1928) have been reported to serve as intermediate hosts of *Acanthocheilonema perstans* (*Filaria perstans*) a non-periodic filaria. *F. perstans* is widely prevalent in tropical Africa, Algeria, Tanis, New Guinea, British Guiana but its principal area

of infection is in and around the Congo basin. The method of invasion of the body of the insect by the filarial nematode is the same as is noticed in the case of *Wuchereria bancrofti* in *Culex fatigans*. Generally within 6 hours the microfilariae have disappeared from the lumen of the stomach and after penetrating the stomach wall they lie in the fat body. The time taken for the microfilaria to undergo complete development is about 7 to 10 days.

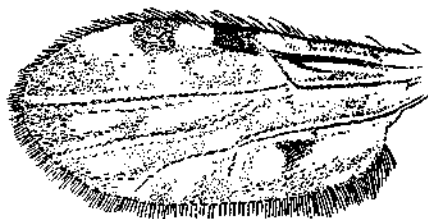


Fig. 80

Wing of *Culicoides oxystoma*.

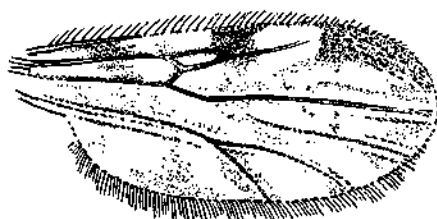


Fig. 81

Wing of *Culicoides anopheles*.

For capturing these and other small midges, a sucking tube as suggested by Buxton (1928), is very useful.

It should be remembered that when these insects are treated with pure carbolic acid for examination, this may cause considerable denudation of small hairs. They should preferably be mounted in gumchloral mixture or in lacto-phenol solution. These and other small midges cannot be preserved in a dry state for a long time and as far as possible they should be mounted permanently.

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Family SIMULIIDÆ

Flies of this family though ethnologically grouped under midges are called black-flies on account of their resemblance to flies. They are dark in colour and are of robust build. They are readily recognised by their general appearance, wing venation and by the annulated antennae which project out like horns of an animal. The antennae are similar in both sexes and each consists of 11 joints. The sexes are distinguished by the eyes which are close together in males and widely

separated in females. The females are vicious biters and suck blood, mostly of cattle. Some species also attack man.

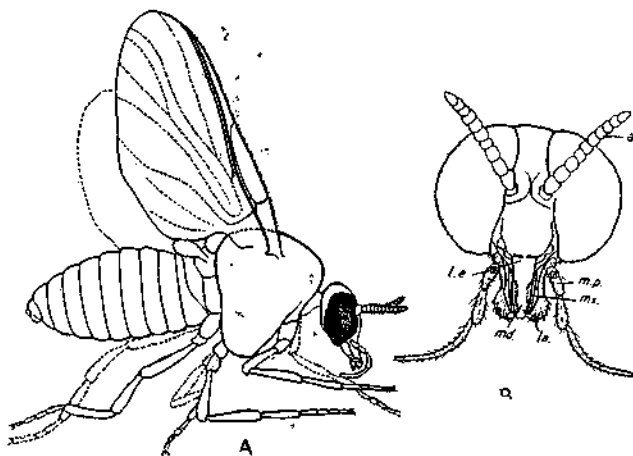


Fig. 82

A. *Simulium indicum*.B. Mouth parts of *Simulium* (after Gibbins).

a., antenna; la., labium; le., labrum-epipharynx; md., mandible; m.p., maxillary palp; mx., maxilla.

The mouth parts in *Simulium* are like those of *Culicoides* but shorter. They consist of the paired mandibles and maxillae, unpaired labrum-epipharynx and hypopharynx, and the fleshy and large labium. The mandible is provided with strong recurved teeth. These are used for cutting and for enlarging the wound. The palpi are 4 jointed.

The wings are large and hyaline. The anterior border is very prominent on account of the presence

of well developed costal and first longitudinal veins. Other veins are poorly developed. There are no scales or hairs on the surface of the wing.

The metamorphosis is complete. The larvae occur in swiftly-running streams usually where there is a strong current and the water is not deep. In India they breed in hill streams. The larvae remain submerged at a depth of one foot or more and fix themselves to stones or plants.

The larva often moves to new positions. At the time of feeding, the food particles are drawn into the mouth with the help of mouth brushes. It is cylindrical in shape and possesses a sucker at the posterior end. It has very strong mouth organs for chewing solids, e.g., crustaceans and other small animals.

Before pupation the larva spins a cocoon inside which the pupa rests. It breathes by means of thoracic gills. The fly emerges while the pupa lies immersed in water and quickly appears on the surface. The larval and pupal stages occupy nearly a month.

The adults are very active on bright sunny days. They are vicious biters. They have been reported as causing loss of live stock from many parts of the world. The dead cattle exhibit reddish spots which are very numerous near the genitalia and on the inner side of the legs.

Simulium flies were at one time suspected of being concerned in the transmission of pellagra but this disease is now known to be of dietary origin. *Simulium damnosum* Theo. has now been definitely proved to carry onchocerciasis to man and possibly also to cattle.

O. volvulus. Onchocerciasis is endemic in tropical Africa. It produces painless tumours on the skin, these being mostly situated on the chest, the crests

of the ileum and the lower limbs. The microfilariae, besides being in the tumours, are always present in the skin in these situations. A fair number of cases show the skin infection without the presence of tumours. Cutaneous onchocerciasis is widely distributed in the central, western and eastern parts of Africa. In 1926 Blacklock discovered the development of *Onchocerca volvulus* in *Simulium damnosum* in West Africa.

O. gibsoni is prevalent in Australia including Queensland. It produces tumours in the hind legs of cattle. It is endemic in Malaya Archipelago, including Java and Siam, producing similar lesions. In southern Rhodesia the infection is very mild.

O. indicum is endemic in India and Ceylon producing nodules in cattle and buffalos. *O. bovis* is found in cattle in France and also in the Argentine Republic. *O. armillata* is extremely common in Egypt and Sudan.

O. gibsoni and other species of *Onchocerca* are possibly transmitted by *Simulium* (Cleland, 1927).

S. damnosum can be easily recognised by the greatly flattened front tarsi and the silvery pubescence of the front tibiae. It is very widely distributed and is found on the banks of streams and rivers.

S. indicum is a common Indian species. Short yellow hairs are present on the abdomen for at least the two front segments.

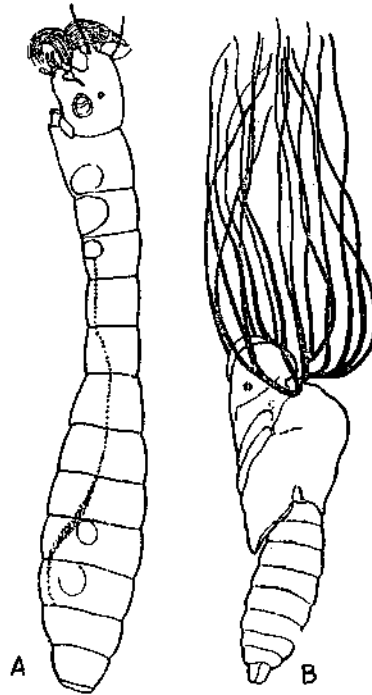


Fig. 83

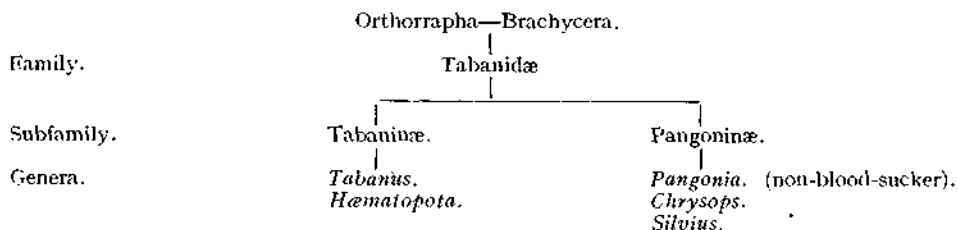
Larva and pupa of *Simulium*. (After Castellani and Chalmers)

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BRACHYCERA

CLASSIFICATION



Family Tabanidae

These belong to Orthorrhapha-Brachycera and comprise a very large group of insects which includes also a large number of families. They are of stout build and only a few are blood suckers. The maxillae are well developed and they all possess rigid maxillary palps. The wing venation is complex. The metamorphosis is complete.

The family Tabanidae includes two subfamilies, Tabaninae and Pangoninae. In the former tibial spurs and ocelli are absent, whereas in the latter these are present. The subfamily Tabaninae includes only two blood-sucking genera, *Tabanus*, and *Haematopota*, while Pangoninae contains *Chrysops* and *Silvius* which are blood-suckers. It is extremely doubtful if the members of the genus *Pangonia* (Pangoninae) are able to suck blood.

In *Tabanus* the 3rd joint of the antenna is provided with a tooth which forms an angular projection. In *Chrysops* the three segments of the antennae are of equal length. In *Haematopota* the wings have characteristic dark spottings and markings.

Genus *Tabanus*. These flies are popularly known as gad-flies. They are large in size and though they are blood-suckers, they do not carry any human diseases. Their chief characters are the following.

Head: Eyes are large and brilliantly coloured in life. In the male the eyes meet in the middle line while in the female there is an intervening space between them on which lies a white band called the callus. Antennae are three-jointed; the third joint is segmented into a number of smaller joints. The third segment is also furnished with a distinct tooth.

The mouth parts lie enclosed in the labium which is comparatively short and is a fleshy structure. The cutting organs are the mandibles and maxillae. The mandibles are well developed and pointed at the tip. The maxillae have serrated edges. Other mouth organs consist of the epipharynx and the hypopharynx.

Wings may be clear or coloured. The venation has a complex arrangement. The abdomen is composed of 7 visible segments.

Life history.

Metamorphosis is complete. Eggs are laid in glued masses on leaves and stems of plants overhanging water surfaces. The larvae on escaping from the egg-shell drop into the water or burrow into wet sand or mud. The larval body is segmented and is very narrow at both ends. In addition to the head, and three thoracic segments, there are 10 abdominal segments. The head carries a pair of antennae, a pair of mandibles and a pair of maxillae with segmented palps. The mandibles are extremely powerful. The breathing tube is attached to the last abdominal segment. A number of body segments bear pseudopods. They thrive on mosquito and fly larvae and other aquatic insects. They sometimes prey on each other.

The pupa is stationary and resembles that of Lepidoptera. The spiracles of the pupa are placed on the thorax. Before pupation the larva crawls out of the water and lies buried in the loose earth.

According to Mitzmain (1913) the whole life cycle in *T. striatus* Fab. takes about 50 days which includes 4 days for the egg stage, 39 days for the larva which moults three times, and 7 days for the pupa.

In the case of the Indian *Tabanus* the larval life lasts 4—6 weeks (Rao and Mudaliar, 1935).

The males in nature derive their nourishment from vegetable juice. A diet of blood is essential for the development of eggs in females.

The adults generally rest on trees. Horses are more particularly attacked than cattle. Man is occasionally bitten.

It is difficult to induce the female to suck blood in captivity.

Disease.

Evans (1880) first drew attention to surra being due to a trypanosome but the precise way in which the infection is commonly transmitted from animal to animal was not known till Mitzmain (1913) pointed out that the common disseminating agent of surra among animals of the equine species in the Philippine Islands is *Tabanus striatus* and the mode of transmission is merely mechanical. This can take place only when the feeding is interrupted. There is no life cycle of the trypanosome in Tabanids. According to Mitzmain, *Stomoxys calcitrans* L. is incapable of transmitting the disease. The findings of Cross and Patel (1921) that *Ornithodoros crossi* Brumpt is capable of transmitting surra have not been confirmed by Yorke and Macfie (1924). In India horse, donkeys, mules and camels are affected; buffalos and cattle seldom manifest any symptom of trypanosomiasis. In India Cross and Patel (1922) found *T. nemocallosus* to be extremely efficient carriers.

Considering the mechanical way in which the trypanosome of surra is carried by *Tabanus*, other blood-sucking flies, e.g., *Musca crassirostris* Stein., *Lyperosia exigua* de Meij., *Stomoxys calcitrans* L., and *Haematopota* sp. may at times also be concerned in the transmission of the disease.

It has also been suggested that *Tabanus* may carry anthrax in the same way.

Kapur (1941) stated *T. orientis* Wlk. can act as a vector of rinderpest. These flies take many blood meals and the feeding is always interrupted.

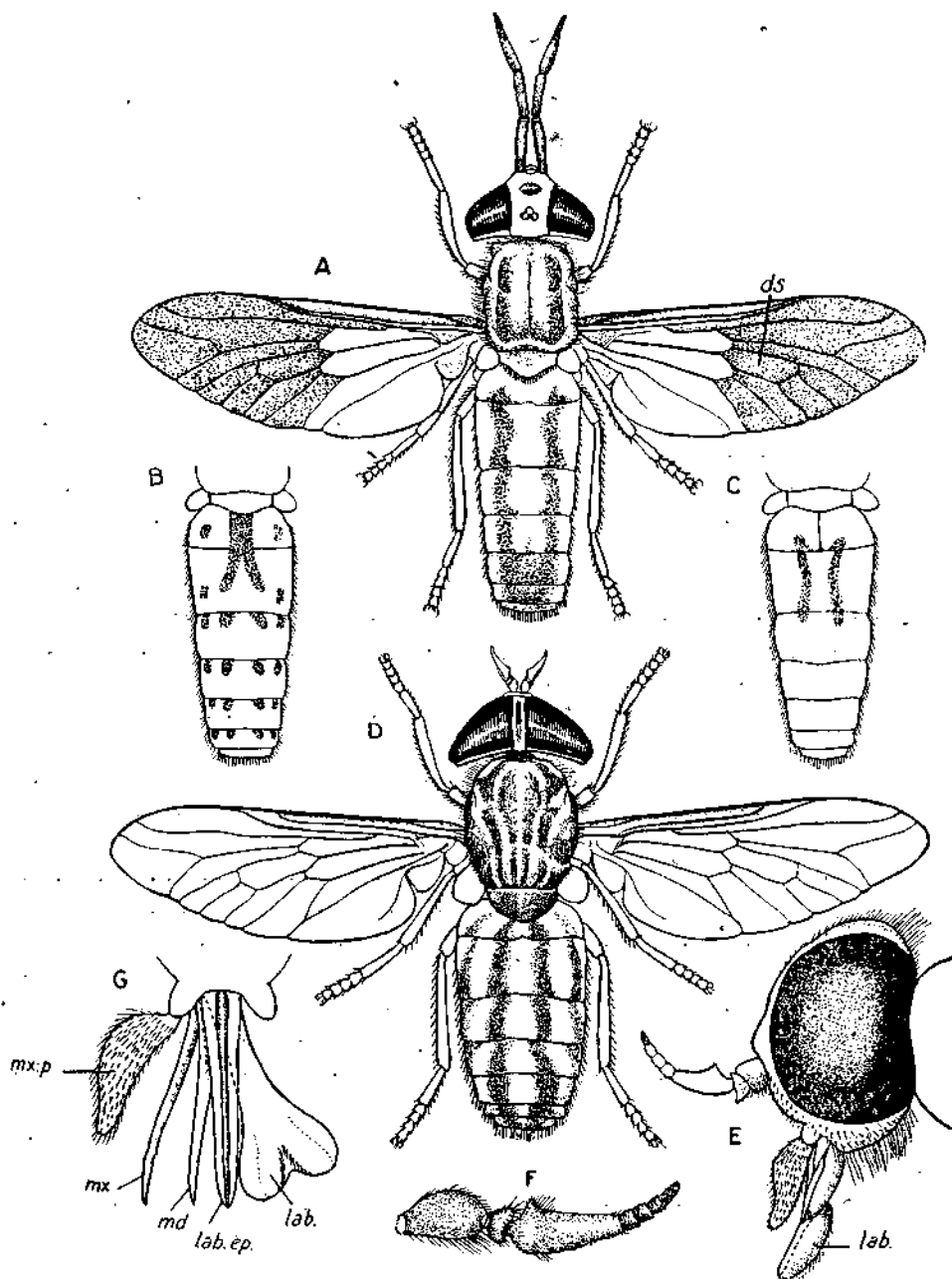


Fig. 84

A. *Chrysops dimidiata*, female. d.s., discal cell. B. *C. discalis*. C. *C. silacea*. D. *Tabanus striatus*. E. Head of *Tabanus*. F. Antenna of *Tabanus*. G. Mouth parts of *Tabanus* of one side only. lab., labium; lab. ep., labrum epipharynx; md., mandible; mx., maxilla; mx.p., maxillary palp. F. Antenna of *Tabanus*.

Common species of Indian *Tabanus*.

T. striatus F. Callus on the forehead has a spindle shaped lineal extension ; abdomen blackish or reddish brown with grey median and lateral stripes almost all the same length.

T. hilaris Walk. Resembles *T. striatus* except that the abdomen with grey median stripe which begins only on the third segment and with lateral stripes which end on the 3rd or 4th segment.

T. albimediis Walk. Forehead has no callus. Abdomen and thorax obscurely reddish brown ; femora reddish brown. The median abdominal stripe composed of almost equal sized spots not very large ; the side spots are large and distinct.

T. rubellus Wied. Resembles *albimediis* except that the abdomen and thorax blackish brown and the femora blackish.

T. speciosus, Ric. Differs from *albimediis* in the median abdominal stripe being composed of spots of unequal size, those on the 3rd and 4th segments being very large and conspicuous. The abdomen and the thorax are reddish brown and the femora blackish.

T. brunnipennis Sehsak. A brown species ; the median abdominal stripe is broad with two round spots on the 2nd segment ; femora reddish ; wings brown on anterior border.

T. ditaeniatus Macq. It is a particularly small species. Forehead has 2 separate callus ; abdomen yellowish with median and lateral blackish and brownish stripes.

T. rufiventris F. (*sanguineus* Wlk.). This is a brown or a blackish brown species. Abdomen is reddish brown or blackish brown with broad white bands and large triangular median spots.

T. orientis. Wlk. Abdomen reddish yellow, darker at apex with narrower yellowish-white segmentations and median spots ; legs black ; tibiae yellowish.

Genus *Chrysops*. They are found all over the world and are smaller and more delicately built than *Tabanus*. There are three ocelli on the vertex. The distal part of the wing is variously coloured in different species. The antennae are long and thin.

The life history is the same as in *Tabanus*. The larvae are found in the mud of swamps. They mostly bite cattle. Some African species, e.g., *C. silacea* Aust., and *C. dimidiata*, v.d. Wulp. bite man freely in the day time and are most troublesome in the late morning and early evening. These flies have been proved by Leiper (1912) to act as intermediate hosts of a filarial disease common in equatorial

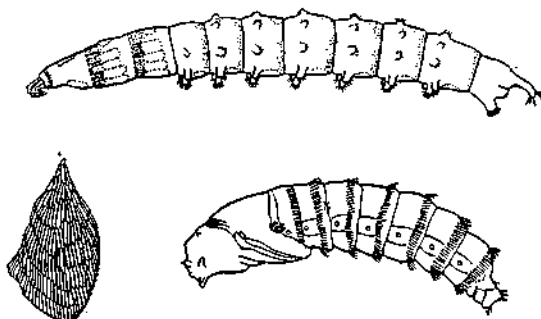


Fig. 85
Egg, larva and pupa of *Tabanus hingi*. (After Austen).

Africa and known as "Calabar Swellings" or fugitive swellings (*Loa loa*). The microfilariae are found in the peripheral circulation during the day time. The infective stage is reached in 10-12 days when the filariae begin to travel towards the head and accumulate at the root of the proboscis and in the labium. They gain exit by the labella. (Connal and Connal, 1922).

The different species of *Chrysops* are distinguished mainly by the markings on the abdomen also by the character of the colouration of the wing.

Both *C. dimidiata* and *C. silacea* possess two broad parallel longitudinal dark brown lines on the dorsum of the abdomen. In *C. dimidiata* the abdomen and legs are yellow. In *C. silacea* the abdomen is reddish and the tip is brown; legs are also reddish.

It has been claimed by Francis and Mayne (1921) and by Francis (1937) that *C. discalis* Will. is one of the transmitters of tularaemia.

Some Oriental Species of *Chrysops*.

C. dispar F.: Wings with a dark transverse band near the middle and anteriorly extending to the apex; a hyaline patch in the lower part of the transverse band; abdomen with a short black bifid stripe on the second and third segments. It is very widely distributed in the Oriental Region.

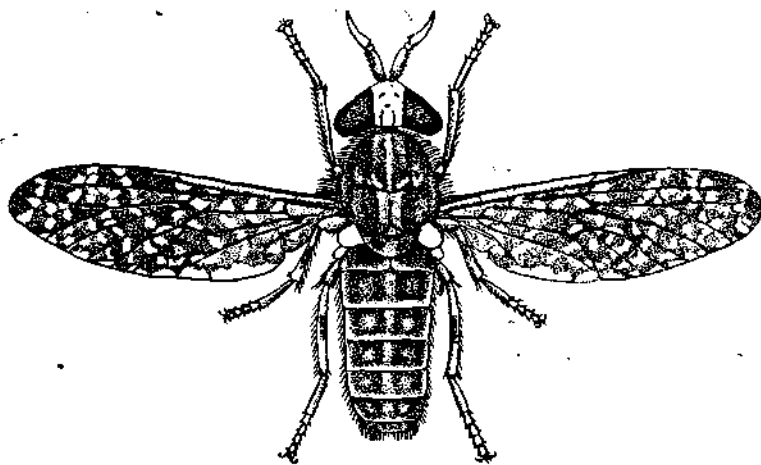


Fig. 86
Haematopota, female.

C. indiana and *C. stimulans* have no hyaline patch in the lower part of the transverse band. In *stimulans* wings have a clear spot in the discal cell and the abdomen is blackish. In *indiana* wings have no clear spot in the distal cell; the abdomen is yellow with a bifid stripe on the second segment.

Genus *Haematopota*. Members of the genus *Haematopota* are readily recognised by the characteristic speckling of the wings together with the presence of ocelli on the frons. They generally attack cattle and man is neglected. One species *H. roralis* Fb. is widely distributed in India, Ceylon and Malaya. The

eggs are laid in long single-tiered clusters of 40-100 on the leaves of rice and other foliage in rice-fields. Incubation period is 4 days. The larvae are aquatic and feed on any soft-bodied animal. They do not attack each other. The larval stage lasts for 5-7 weeks. Pupation takes place in rather drier soil but close to the larval habitat. The pupal period lasts about 7-9 days. The flies are most abundant in July-August (Isaac, 1932).

Genus *Sylvius*. They are widely distributed in the tropics. They are medium sized flies, and some of them may resemble *Tabanus*. The wings are hyaline. The third segment of the antenna has a basal tooth; ocelli and tibial spurs are absent. The proboscis is, however, considerably shorter. Only the females suck blood.

Genus *Pangonia*. The marked feature in these flies is the presence of an exceptionally long proboscis which projects horizontally. The precise economic importance of *Pangonia* seems to be doubtful. According to many it lives on liquid diet other than blood. The long proboscis is used to extract the nectar from flowers. The flies are found chiefly in forest areas.

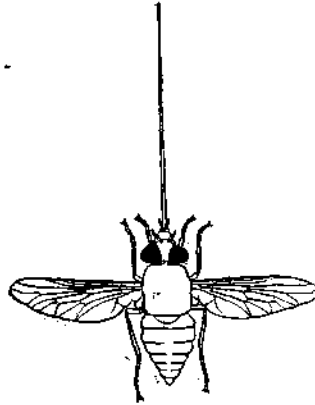


Fig. 87
Pangonia longirostris, male.

Diptera Aschiza. (Hover flies).

These insects do not possess frontal suture and in this respect they differ from true flies. The only importance which can be assigned to this group of insects is that the larvae of one species are known to find their way into human intestines possibly with drinking water and produce unpleasant symptoms such as abdominal pain, vomiting etc., at times simulating appendicitis or intestinal obstruction.



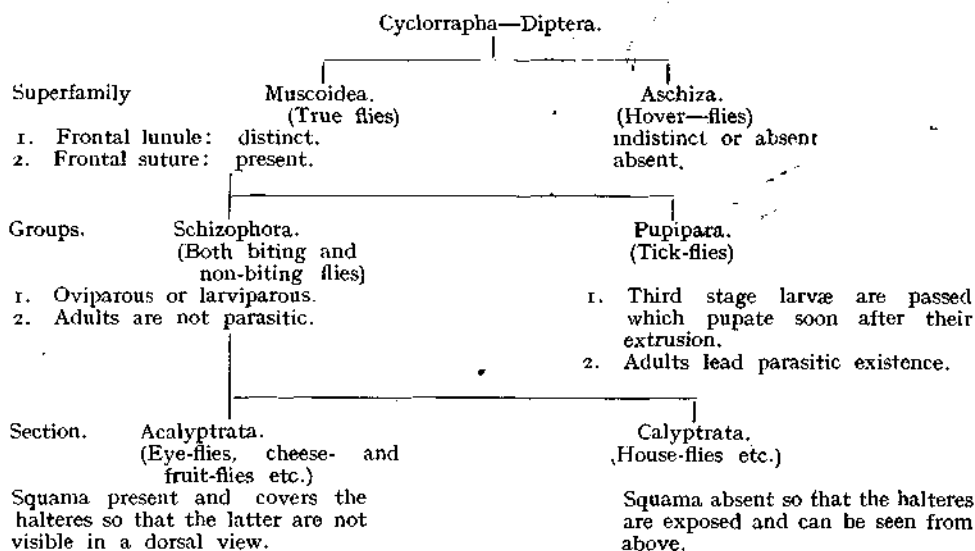
Fig. 88
Rat-tailed larva of *Eristalis*.

The insects found in gardens and which closely resemble wasps in external appearance and are known as hover-flies, belong to this group. The larvae of certain species are aquatic and on account of the presence of a long breathing tube, they are known as rat-tailed larvae. They are found in extremely foul water and it is surprising how they can gain access into human intestines. No such case, however, has yet come to our notice in India.

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CYCLORRAPHA



Superfamily MUSCOIDEA.

This includes true flies and some of them are of considerable economic importance to man.

The house-fly is taken as a typical example of an insect belonging to the Order Diptera. It has a retractile proboscis, it sucks liquid food and has a complete metamorphosis. The different stages in its life are egg, larva, pupa and adult. The larva is maggot-like and the pupa develops inside a hard chitinous shell from which the adult fly escapes.

A short description of the structure of the fly and its different stages together with its habits is given below.

The body of an adult fly consists of head, thorax and abdomen. The neck is mobile.

Head. (1) Eyes are large and compound. In the common house-flies the sexes can be distinguished from the approximation of the eyes; these are close together in males but are divergent in females. The vertex or frons is the space between the eyes. (2) Simple eyes; or ocelli, three in number—may or may not be present; (3) Antenna: 3 segmented; the last segment is the longest from which arises a long stylet called the arista; the arista may be simple or branched. (4) Proboscis (or labium) which is retractile in non-biting flies and stiff in biting flies. Maxillæ and mandibles are wanting in all flies though the maxillary palps are well developed. At the distal end of the proboscis lie the labellæ.

The proboscis of the house-fly consists of three parts named from 'above' downwards, rostrum, haustellum, and oral disc. The rostrum can be voluntarily

withdrawn or extended. The extension of the rostrum is brought about by the distension of the large air sacs which are contained within it. The rostrum is more

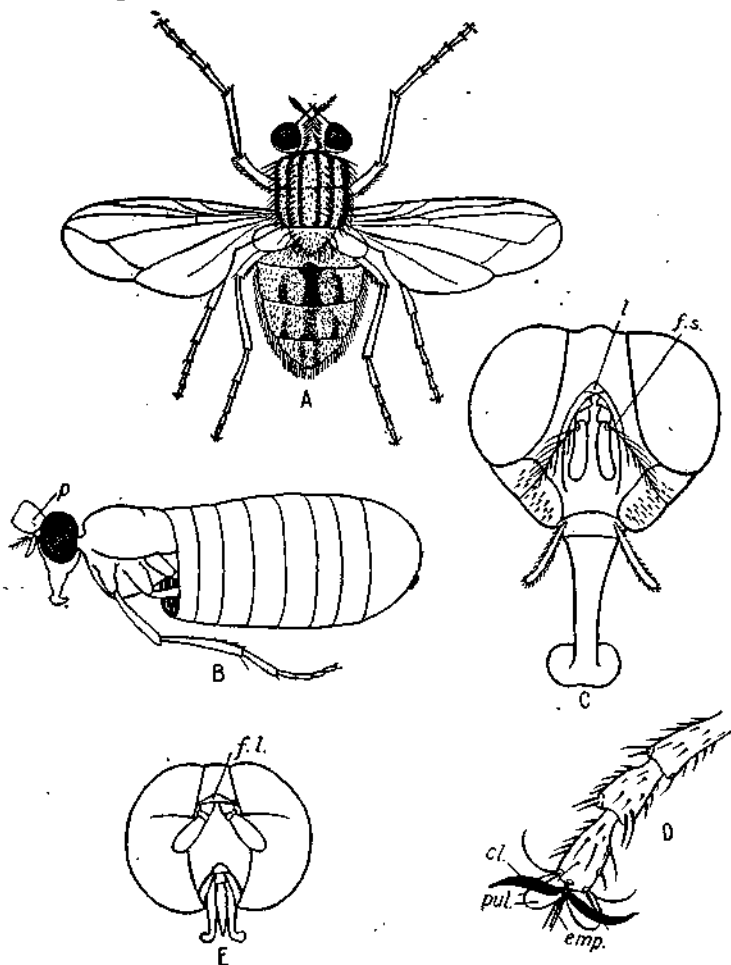


Fig. 89

- A. House-fly; B. Emergence of fly from the puparium. p, ptilinum.
 C. Head of house-fly. f.s., frontal suture; l., lunule.
 D. Foot of house-fly. cl., claw; pul, pulvillus; emp, empodium.
 E. Head of Aschiza. f.l., frontal lunule.

dilated than the haustellum and consists of a thick membrane called the fulcrum which has a triangular outline, the cone of the triangle pointing downwards. The pharynx runs along the ventral wall of the rostrum. To the lower part of the rostrum are attached the two maxillary palps.

In a deep groove in the haustellum lie the labrum—epipharynx, and the hypopharynx. Through the hypopharynx runs the terminal part of the salivary duct. The labrum-epipharynx is dagger-shaped and lies dorsal to the hypopharynx. The food channel is formed by the apposition of the labrum-epipharynx and the hypopharynx.

The oral disc consists of 2 labellae, the inner surfaces of which are grooved by a large number of chitinous channels, the pseudotracheae, running transversely across the labellum almost parallel to each other. Each pseudotracheal ring is incomplete; one extremity is flattened and the other is forked or bifid. The rings are arranged in such a manner that along each side of the central fissure the bifid extremity of one ring alternates with the expanded extremity of the next ring. The area enclosed between the forks of the bifid extremity of a ring has been termed the "interbifid space" by Graham-Smith (1911). Food really enters the pseudotracheae through the spaces between the bifid extremities of the rings (or interbifid spaces) and not through the continuous zigzag fissure which is extremely narrow and is probably closed during the act of feeding. The size of the solid particles which can pass in the mouth is regulated by the width of the interbifid spaces. Certain relatively large objects such as the ova of tapeworms, too large to pass through the filter, may occasionally be swallowed. Such objects probably pass directly into the mouth. The liquid food is sucked into the pseudotracheae and drawn through the collecting channels into the mouth. Each collecting channel opens into the corresponding gutter between the prestomal teeth in a remarkable manner.

The prestomal teeth are chitinous prong-like processes free at their distal bifid extremities but with their proximal portion embedded in the dental area of the pseudotracheal membrane. The teeth are used for scraping solid food, *e.g.*, sugar (Graham-Smith, 1930).

When at rest the oral surface of the labellar or oral lobes are in opposition, but during feeding they are spread out over the surface of the food so as to form an oval disc.

The labial salivary glands lie at the base of the labella. Each consists of a large number of glandular cells. They open externally by a number of small ducts on the labellar surface. It is thought that the function of the labial saliva is to seal the longitudinal fissures of the pseudotracheae by its viscid secretion during the act of feeding.

Thorax: The thorax is mainly composed of the mesothorax; the pro and the meta-thorax are poorly developed. A transverse suture separates the mesonotum into prescutum and scutum. The scutum is further divided by a suture into

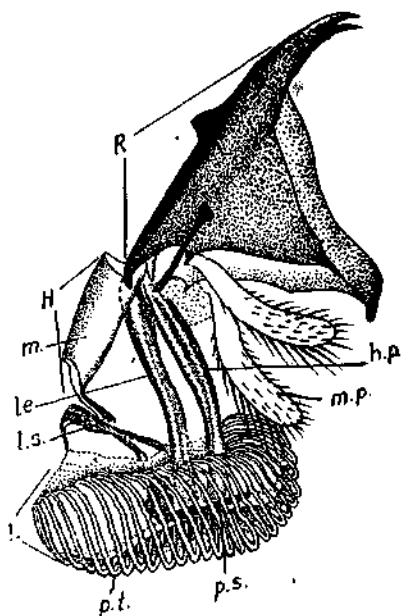


Fig. 90

Mouth parts of a fly.

R. rostrum; H. haustellum; L. labella.
m. mentum; le. labrum-epipharynx; hp.
hypopharynx; mp. maxillary palp; l.s.,
labial sclerite; p.t., pseudotracheal tube;
p.s., prestomal sclerite.

scutum proper and the scutellum which lies posteriorly and overhangs the metathorax. There are numerous bristles on the dorsum of the thorax; the most

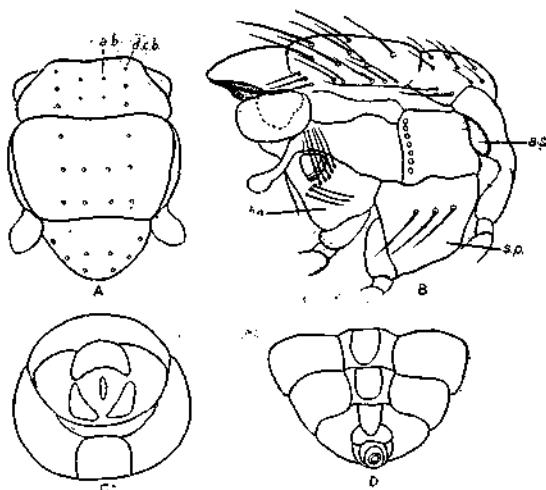


Fig. 91

A & B. Thoracic chaetotaxy of fly. C & D. Ventral view of the last segment of the abdomen of *Chrysomya megacephala*. C, male; D, female.

a.b., acrostichal bristles. d.c.b., dorsocentral bristles. as, anterior spiracle. s.p., sternopleura. h.p., hypopleura.

important ones are (1) a row of acrostichal bristles and a row of (2) dorso-central bristles on each side of the middle line. On the pleuron of the mesothorax lie a number of sclerites, the important ones being sternopleura and hypopleura, each bearing well developed bristles.

The thorax bears two pairs of spiracles, three pairs of legs, and a pair of wings.

Each leg is composed of (1) coxa (2) trochanter (3) femur (4) tibia and (5) five tarsal segments. The last tarsal segment ends in a pair of claws. On the inner side of each claw lies a foot—pad or cushion covered with hairs and called pulvilli. Between the pulvilli

lies a long bristle called the empodium.

Wing. The venation of the wing of the house-fly is comparatively simple. There is no branched vein. The 4th longitudinal vein bends distally in a characteristic manner so as nearly to enclose the first posterior cell.

Near the base of the wing is a membranous expansion attached to the side of the thorax just posterior to the attachment of the wing. This is known as the squama. In house-flies it conceals the halteres when examined from the dorsal aspect.

Abdomen: There are 8 abdominal segments in the male and 9 in the female fly. Among them only four segments are visible. These are the 2nd to 5th. The first segment is very much reduced. Segments 6 to 8 in the male are also reduced. In the female segments 5 to 9 form the tubular ovipositor, which, in repose, is telescoped within the abdomen. It is extended only at the time of oviposition. The tergal plates of the abdomen are well developed whereas the sternal plates are greatly reduced.

Alimentary canal. The alimentary canal is divided into (a) foregut, (b) midgut, and (c) hindgut. In the foregut is included the pharynx, oesophagus, oesophageal diverticulum and proventriculus. The midgut consists of the stomach and intestine where active digestion of food takes place. The intestine, rectum and malpighian tubules form the hindgut.

(1) The mouth lies between the labellae where the pseudotracheal collecting channels lead into the food channel. The common salivary duct which passes through the hypopharynx opens into the ventral surface of the buccal cavity.

(2) Pharynx: its function is to pump liquid food and forms the first part of the alimentary canal. It extends upwards from the mouth along the posterior aspect of the fulcrum and terminates above at the proximal end of the latter. (3) Oesophagus: takes its origin at the proximal end of the fulcrum and passes through the cephalic ganglion and then the neck; it ends in the anterior thoracic region at the proventriculus. (4) Oesophageal diverticulum or crop. The reservoir or crop is a large distensible bilobed sac lying in the abdomen. When distended with liquid food, it becomes reniform. The duct of the crop begins and opens at the posterior end of the oesophagus where the latter terminates and opens into the ventral side of the proventriculus. The special function of the crop is to store liquid food. When the alimentary canal is empty, food is rapidly expelled from the crop. It is filled with great rapidity.

All food materials ingested by the fly first pass through the oesophagus into the crop and from there through the proventriculus into the intestine. From time to time the fluid contained in the crop is regurgitated into the mouth and appears as a vomit drop.

(5) The proventriculus is formed by the invagination of the foregut into the midgut, and acts as a valve between the midgut and the foregut. (6) The midgut or stomach is a long coiled tube and lined with the peritrophic membrane which also imperfectly lines the inner wall of the hind-gut. (7) The hind-gut is comparatively short. (8) The rectum has 4 papillae, their function being to absorb water from the rectum and probably also excrete urea from the blood. (9) Malpighian tubules: two on each side and arise from a common stalk which soon divides.

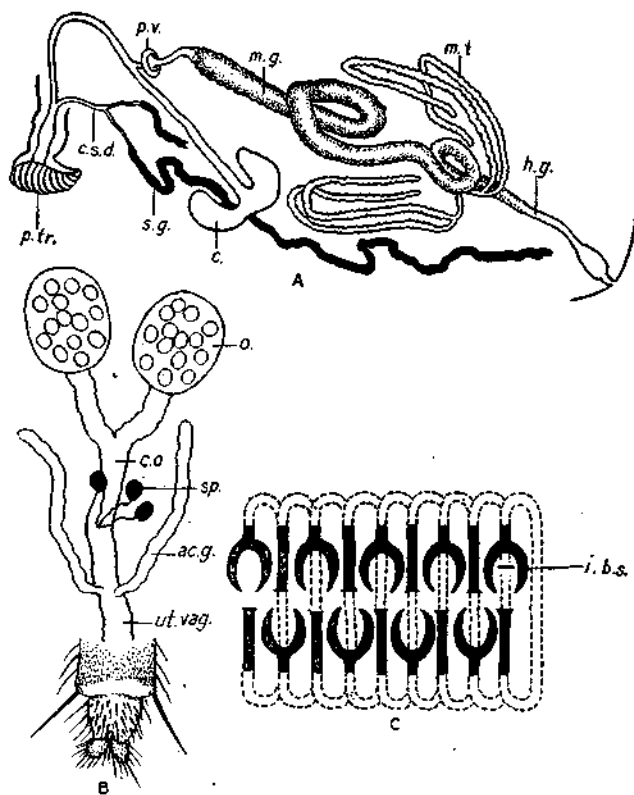


Fig. 92

- A. Alimentary system of house-fly.
 c, crop; c.s.d., common salivary duct; h.g., hindgut; m.g., midgut; m.t., malpighian tubules; p. tr., pseudotracheae; p.v., proventriculus; s.g., salivary gland.
- B. Generative organs of a female house-fly.
 ac.g., accessory gland; c.o., common oviduct; o., ovary; sp., spermatheca or vesicula seminalis; ut.vag., utero-vaginal tube.
- C. Pseudotracheal tubes showing interbifid space (i.b.s.)

They are very long, convoluted and bound up with the adipose tissue containing the fat body. They function as renal organs especially serving to excrete urea and other waste products from the adipose tissue and the blood.

Salivary glands. To avoid confusion and in order to distinguish them from the labial glands these are designated as lingual salivary glands. They are two extremely long fine tubes of uniform length except at the distal end. They are considerably longer than the total length of the body. The small salivary valve is situated within the common duct a short distance from where it enters the hypopharynx.

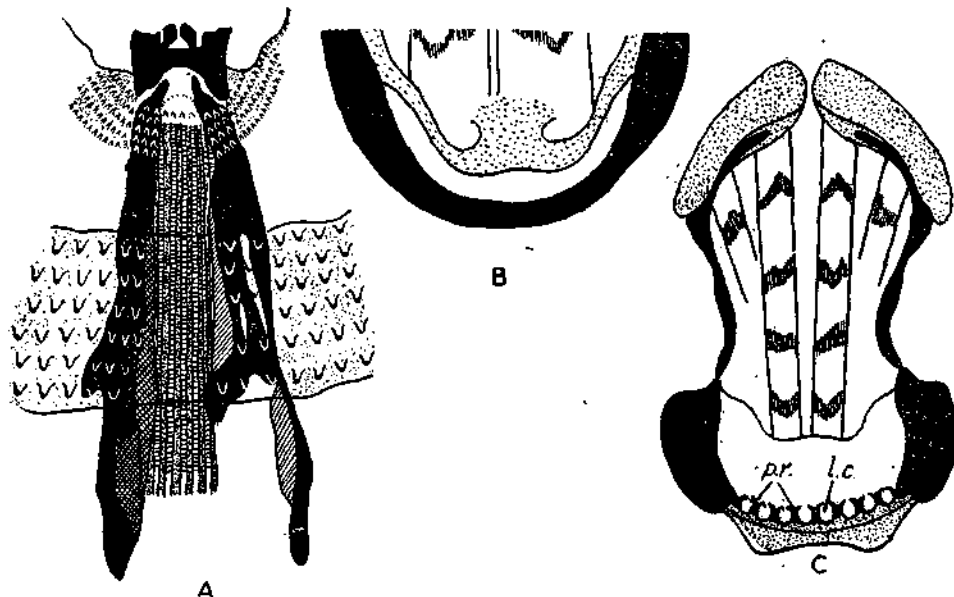


Fig. 93

A. *Calliphora erythrocephala*, showing the pharyngeal ridges on the ventral side of the pharynx of the larva. (After Roy). B. Transverse section through the pharynx of *Gasterophilus equi (intestinalis)* larva showing the absence of ridges on the floor of the pharynx. (After Roy). C. Transverse section through the pharynx of the larva of *C. erythrocephala* showing the pharyngeal ridges (p.r.) and the longitudinal channels (l.c.) where the solid particles are usually caught. (After Roy).

Respiratory system. The respiratory system is characterised by the presence of air sacs developed in connection with tracheal trunks in the head, thorax and abdomen. These large tracheal trunks open externally on the thorax and abdomen. The thoracic spiracles are two on each side; the abdominal spiracles differ in number in the two sexes; in male there are seven pairs, in the female five. It is extremely doubtful if the abdominal spiracles are functional like the thoracic ones.

The reason of the existence of air-sacs in insects has not been properly understood. It may be a means of lowering the specific gravity of the insect or the volume of the tidal air may be increased by providing regions in the tracheal system which can be readily compressed and distended.

Reproductive System.

Female: (a) a pair of ovaries containing a large number of ovarioles; (b)

short oviducts ; (c) common oviduct ; (d) vagina ; (e) spermathecae, three in number ; their common duct opens in the common oviduct ; (f) one pair of accessory glands ; these lie on either side of the common spermathecal duct.

Male. (a) a pair of brown pyriform testes ; (b) vasa deferens ; (c) a long ejaculatory duct opening into a small ejaculatory sac and continued into a aedoeagus ; (d) a pair of accessory glands.

Larva.

It is a footless, hairless maggot ; the body consists of head, three thoracic and 9 abdominal segments. The anterior end is tapering and the posterior is much broader and truncate. The head is not demarcated from the rest of the body. Sometimes projecting from the cephalic end of the body is a pair of highly chitinised hooks. The antennae and maxillary palps are present on the first segment. The ventral and ventro-lateral surfaces of the oral lobes are traversed by a number of channels which resemble the pseudotracheae found on the labella of the adult fly. Eyes are absent.

Three pairs of setiferous papillae present on the ventral surface of the thorax in connection with the imaginal disks of the legs have been described by Keilin (1915). This indicates that the atrophied legs of the maggot have been transformed into sensory organs.

On the second segment lie the anterior spiracles and the posterior spiracles are situated in the middle of the truncate end of the last segment. The anterior spiracles consist of a number of finger-like processes. The posterior spiracle is round and the openings are slit-like. On the ventral surface of the last six segments are pads which are used in locomotion.

The cephalopharyngeal skeleton lies well within the oral lobe of the larva. It consists of 3 pieces of sclerites ; these are from before backwards, mandibular, hypostomal and pharyngeal. On the ventral surface of the last-named sclerite lies the pharynx the wall of which in some species may be ridged.

Pupa.

The pupa is barrel-shaped. The colour is dark brown. The last larval skin is not cast off in flies but hardens to form the outer covering or puparium inside which the pupa develops. The puparium shows almost the same outward structures as the fly larva. Thus antennae, the maxillary palps and the anterior spiracles are visible while the posterior spiracles are also noticed on the last segment.

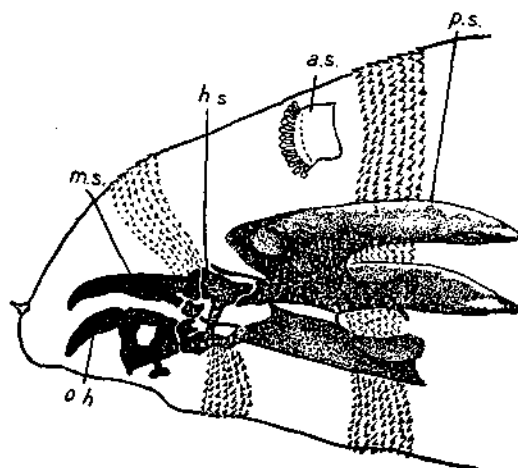


Fig. 94
A. Anterior part of a fly larva showing cephalopharyngeal skeleton and anterior spiracle. a.s., anterior spiracle ; h.s., hypostomal sclerite ; m.s., mandibular sclerite ; o.h., oral hook ; p.s., pharyngeal sclerite which is deeply incised posteriorly.

Life History.

The egg of the house-fly measures about 1 mm. in length, is creamy white in colour, roughly oval, and presents a highly polished surface. The anterior end is narrow and the posterior is broader. On the dorsal surface of the chorion are two longitudinal ridges with a concave area between them.

Temperature has a marked effect on the developmental cycle of the fly and a sudden change from heat to cold will materially prolong any of the stages.

During the most favourable time in summer in this country eggs may hatch within 3 hours after they are deposited. In the winter this period is extended to more than 2 days. On an average eggs do not take longer than 48 hours to hatch.

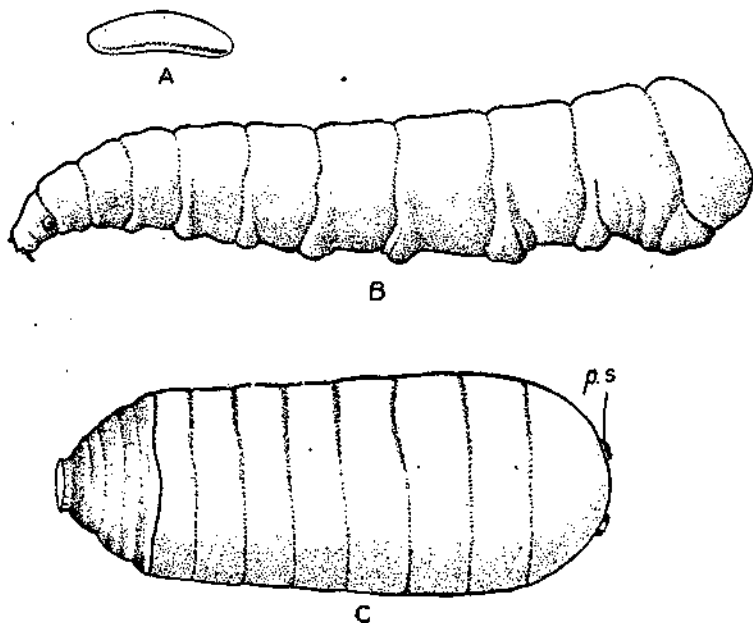


Fig. 95
Egg, larva and pupa of house-fly.
A, egg; B, larva; C, pupa; p.s., posterior spiracle.

Eggs of house-flies are laid in any fermenting material of organic or vegetable origin, or in refuse that is likely to ferment, *e.g.*, garbage, human faeces, horse-manure, kitchen refuse etc. Other flies select cow-dung for oviposition, or a carcass. Some have a predilection for dead birds while there are some which oviposit in human and animal wounds. Thus it will be seen that different species have different breeding habits.

The eggs are seldom scattered on the surface but are carefully deposited one on top of another. By means of its long ovipositor the fly generally deposits its eggs in dark places away from light and some distance from the surface.

On an average a single *Musca vicina*, *M. nebulosa* and *Chrysomya megacephala* can deposit in summer, 97, 75 and 182 eggs respectively, whereas in December the respective numbers are reduced to 72, 64 and 166. The ovaries completely discharge their contents when a batch of eggs is laid, and after a single act of

oviposition, further deposition of eggs does not take place within 3 days which is the normal time for the development of eggs and their maturation within the ovary (Roy, 1938).

The larva is about half-an-inch in length ; it is a footless, hairless maggot and is very active. The fleshy pads which lie on the ventral surface of the body help the larva in its progression. It resents light, either natural or artificial. The larva is a fluid feeder.

The floor of the pharynx in certain Diptera larvae is provided with longitudinal ridges. The nature of the ridges varies in different types of larvae and Keilin (1912) considers this to be an adaptation to the manner of life. In saprophagous larvae which feed on decayed vegetable matter or the excrement of herbivorous animals, the buccal armature is similar to that of *Calliphora* or *Lucilia*. These have a number of longitudinal ridges projecting into the lumen of the pharynx. Parasitic larvae, on the other hand, have a smooth pharyngeal wall without any ridges. This type of pharynx is seen in larvae of *Chrysomya bezziana*, *Cochliomyia americana*, etc. (For diagrams see fig. 93).

The function of the ridges seems to be connected with filtration in the nature of the separation of the large particles of solids from the liquid food ; the former are lodged in the spaces enclosed by the ridges and remain there during the rest of the larval period. The solids thus caught in the ridges are found in the same situation in the adult stage and these gradually pass out from the alimentary canal within 12 to 16 hours after emergence of the fly (Roy, 1937).

The larva moults twice. The first stage larva has no anterior spiracles. The third stage larva has the habit of migrating. They may even burrow to a depth of 2 ft. in loose earth before they pupate. This migratory habit, which is so much in evidence during the prepupal state of the house-fly, has long been known. As pupation draws near the larvae pass from the very moist regions of a manure heap and seek the comparatively dry outer regions. If no such places are in the heap, they will leave it to pupate in the ground or in loose material of any kind, even at some distance from the breeding place. This habit offers an important point of attack in the control of this pest. Farm refuse and manure may be placed in a barrel in the bottom of which several holes are made, with the result that on the following day thousands of maggots will be found in water placed in a tub beneath. In this way perhaps 70 per cent of the maggots can be destroyed. Larvae kept in horse-manure, sterilised from time to time, will fail to grow. The length of life of the larva varies according to temperature. The earliest recorded in Calcutta is between one and a half and two days. Before pupation the larva does not feed but empties its alimentary canal and ceases activity. It contracts and transforms itself into a barrel-shaped object ; its colour gradually changes from white to brown and finally to chestnut colour. The last larval skin is not cast off but hardens to form the puparium.

Under the conditions present in the tropics, the pupal stage does not last more than 4 to 6 days. In cold places this stage may be considerably prolonged.

When the fly is about to emerge, air is forced into its anterior part and an air-cushion or ptilinum appears on the forehead. The anterior part or the cap

of the puparium is pushed off, a circular slit appears through which the fly escapes leaving behind the nymphal sheath, which lines the inside of the puparium. In course of time the ptilinum retracts giving rise to a rhomboidal scar called lunule, and a frontal suture; the latter is absent in many flies, *e.g.*, *Aschiza*.

The ptilinum not only helps the fly in escaping from the puparium but also in forcing its way through soil when the pupating larvae are buried in the earth.

In the tropics during summer a fly is able to complete its cycle within $5\frac{1}{2}$ to 6 days. At other times this period is correspondingly increased depending primarily on the temperature and secondarily on the quantity of food available for its growth.

House-flies are usually more attracted to sugar than to other types of food although they are omnivorous in nature. The fly feeds intermittently and at the same time voraciously. It has the habit of sitting on filthy substances such as excreta, sputum, sores etc., and also on food. After feeding, the fly cleans its head and proboscis with its legs. From time to time it vomits or regurgitates its food from the crop, the regurgitation appearing as a minute drop of fluid hanging from the oral lobes. The vomit also contains a drop of saliva. Some time the vomit drops are deposited like faeces upon the surface on which the fly is sitting. Although it has been said that their colour and characters are different, it is not easy to distinguish between the two, vomit and faeces. It is thought that the vomit drops are intended to soften solids, such as sugar, but even after 24 hours' starvation when no vomit drops are seen on the labellae, the flies will still feed voraciously on sugar. Perhaps the saliva helps such solids to be converted into the liquid form. The presence of a large amount of fluid in the crop always leads to an increase in regurgitation. The fly has also the habit of constantly defecating. Under normal conditions, flies fed on coloured food will continue the defecation of coloured excreta for at least 48 hours. If, however, the flies have been starved before ingesting the coloured food, this food may appear in the faeces within 2 hours, and all the coloured food will be expelled within 6 hours. The emptying of the crop normally takes place within 48 hours, but if the fly is placed in cold surroundings it is possible to detect the coloured food in the crop even after 72 hours.

Copulation takes place within 24 hours after emergence. Once fertilised, it is sufficient for her whole life time.

Protein food is necessary for the development and maturation of the egg.

The adult flies are generally not capable of dispersing to a distance greater than a quarter of a mile.

It is doubtful if adult flies live longer in nature than inside the laboratory cage. They have a large number of enemies and fall a ready prey to the latter when the flies rest at night. Spiders and lizards may be counted as their most deadly enemies. Flies do not generally live longer than 15 days in the summer and 25 days during winter. Provided there is no dearth of the proper type of protein food a fly may deposit a batch of eggs every four days.

The house-fly cannot pass the winter in the adult state when exposed to outdoor conditions. Its larval and pupal stages are greatly extended and are passed in or under large manure heaps. It is possible that the method of overwintering by continued breeding is much more widespread than is commonly realised.

Parasites of flies.

(1) *Habronema muscae*, Carter. The embryos are passed with the alimentary dejecta of horses and find their way into the alimentary canal of fly larvae. The young worms reach their final stage at about the time the flies emerge in the winged state. In the flies, the parasites are commonly found in the head and frequently in the proboscis, but they also occur in the alimentary canal and in the thorax. (2) *Allantonema stricklandi* Roy and *A. muscae* Roy. These nematodes are found in the haemocoel of *Musca vicina* and *M. nebulosa*. (3) *Herpetomonas* species. (4) Different species of Hymenoptera. A very large number of Hymenoptera of Superfamily Chalcidoidea are known to parasitise Muscoid puparia, and to act as natural enemies of the latter (Roy and Siddons, 1939). (5) A fungus, *Stigmatomyces baeri*, is a common parasite of adult flies.

Flies and Human Diseases.

The habits of flies always compel them to pick up disease germs from sores, ulcers, stools etc., and carry them to man. Such habits are peculiar to flies and the more opportunities they have of coming in contact with man, especially with his food and drink, the more is the potential danger arising from their presence in the household and in camps. The habits which are particularly dangerous from the point of view of the carriage of disease germs are the following:

(a) The fly is an intermittent feeder; it constantly defecates and vomits every now and then.

(b) It feeds indiscriminately on filth and articles of food intended for human consumption such as sugar, sweets, milk etc.

(c) It has a restless nature. Whether for the purposes of feeding or not it will alight on any article making no difference between food and filth. It has a tendency to settle on sputum, sores, stools, etc., on which it also feeds.

(d) During the larval stage it may feed on pathogenic organisms which sometimes may be carried over and may be discovered in the excreta of the adult fly.

Such habits of house-flies leave no room for doubt that they are highly conducive to the spread of pathogenic germs in general and not to any one in particular, though they play an important part in the spread of intestinal diseases of man.

The different ways by which domestic flies can propagate disease-germs are:

(i) by passing ingested pathogenic germs, e.g., of cholera, typhoid and dysentery etc., sucked up from the excreta of man, tubercle bacilli from sputum, or lepra bacilli from leprotic ulcers;

(ii) by carrying them on the external surface of the body, e.g., on hairs and bristles, wings, foot-pads, proboscis etc., and

(iii) by vomiting the ingested materials.

It will thus be clear that the fly spreads disease organisms by acting merely as a mechanical agent.

The scientific aspect of the problem of flies and diseases is, however, exceedingly complicated. The organisms responsible are of microscopic dimensions and may be either bacteria, protozoa, or helminth eggs, e.g., of tape-worms and

round-worms of man. Though conjectures can be made in regard to the carriage of these organisms by flies, the final problem, that of fixing the responsibility for a particular disease on flies, is perhaps the most difficult of all. In suspected fly-borne diseases, numerous other factors have to be considered, *e.g.*, the food may be infected through the agency of wind, dirty utensils, soiled fingers or contamination of the drinking water. Thus all the evidence must be carefully analysed before any final conclusion can be reached.

The evidence of the fly being responsible for the spread of disease organisms has been obtained mainly from three sources:—

1. Epidemiological or general observations during epidemic outbreaks.
2. Experimental evidence of the infection of flies with pathogenic organisms and the subsequent recovery of the latter from infected insects.
3. Recovery of specific disease-producing organisms from wild flies.

It may be pointed out that no proof has yet been obtained of the multiplication of disease germs in either the crop or the intestine of the fly.

Typhoid fever. Flies have long been associated with the cosmopolitan scourge of typhoid fever. In fact Howard suggested the name of 'Typhoid fly' for the common house-fly, *Musca domestica*, of the temperate regions. During the South African war, typhoid fever accounted for 30 per cent. of the mortality and it was thought that flies were responsible for the spread of the disease by conveying the typhoid bacillus from the insanitary latrines to the food of the soldiers. Similarly in India medical opinion has often expressed the idea of close association of flies with typhoid, based on the correlation of seasonal prevalence of house-flies and typhoid outbreaks in military camps and cities.

Summer and infantile diarrhoea. On the basis of epidemiological investigations it has been suggested that house-flies play a significant role in the dissemination of summer diarrhoea. It is possible to obtain non-lactose fermenting types of organisms in the intestine of flies similar to those found in the faeces of children during the time when this disease is prevalent.

Cholera. Gill and Lal (1931) in discussing the epidemiology of cholera in the Punjab from 1924-29, thought that water was not apparently responsible, since the wells were systematically disinfected. Infection by wind was thought possible since dust storms preceded a rise in the daily number of cases. But the outbreaks were most intense and the incidence most high in the neighbourhood of fly-infested nightsoil dumps. Gill came to the conclusion that the house-fly was the agent in the spread of the disease.

Gill and Lal (1931) made laboratory investigations on the house-fly as an insect host for the cholera vibrio. They found that the cholera vibrio survives for at least five days in the gut of the fly and during this period it is capable of infecting milk by means of its proboscis. The bacilli are found in its faeces for a period of up to twenty-four hours.

Dysentery. With regard to dysentery, it has been conclusively proved that flies are capable of ingesting and passing viable cysts of *Entamoeba histolytica*, and also the bacteria, *B. shiga* and *B. flexner* responsible for the two forms of bacterial colitis.

Intestinal worms. Experiments in Calcutta with house-frequenting flies suggest that while *Musca vicina* and *M. nebulosa* can ingest only eggs of the size of hook-worm and thread-worm, *Chrysomya megacephala* and *Sarcophaga ruficornis* can in addition swallow the larger eggs of tape-worm and round-worm.

Other diseases. The possibility that a large number of other diseases, e.g., leprosy, anthrax etc., may be spread by house-flies, exists but future observations alone can definitely assess its extent. Houseflies are capable of absorbing enormous quantities of Hansen's bacilli (Lamborn, 1935). In the case of yaws, Castellani (1907) found *Treponema pertenue* in *Musca domestica* after they had fed on scrapings in yaws lesions.

Fly Prevention.

The prevention of house-frequenting flies is indeed a difficult matter but it is always possible to reduce their number to an appreciable extent. In combating flies and diseases carried by them our aim should be to eliminate the breeding sites altogether. As far as possible conditions should be made unfavourable for the flies to deposit their eggs. The eggs and larvae wherever found should be killed and the adult fly destroyed.

In Europe horse manure has been looked upon as particularly responsible for fly breeding. In India, on the other hand, where horses are so few in comparison with temperate regions, horse manure does not constitute a very important source of their breeding though it is admitted that the flies will readily breed in it if they get the opportunity. House-flies in India have a wide choice of breeding materials and in large cities these are garbage, refuse of all sorts, ash-pits, and human and animal excreta. Human excreta and conservancy trenches are favourite breeding sites for most of these flies. City refuse dumps and incinerators, if badly worked, are fertile breeding grounds. Offal and undigested materials from the stomach of ruminants obtainable in slaughter houses also yield a fair number. Cow-dung, both in bulk and in patches, cannot be ignored and some of the domestic flies are attracted to it.

Other flies in addition to house-flies breed almost exclusively in human excreta, in bulk and in single deposits. The blue-bottle breeds in cesspits containing almost liquid faeces. This fly is perhaps least conservative in breeding habits, for it also readily breeds in putrifying meat and in cadavers. The flesh-flies breed to a large extent in putrifying animal remains.

Thus almost all fermenting and excrementous organic matters will breed flies. It is no exaggeration to say that fly-breeding is an excellent index of the standard of public hygiene, and in this respect some of India's largest cities are notoriously deficient.

Armed with the knowledge of the breeding and feeding habits of these flies, we are now in a position to discuss effective control measures against them.

Prevention of egg-laying by proper disposal of refuse and garbage.

The first step in this respect is to know how these flies can be prevented from egg-laying. Here the individual householder and the owner of a shop or food-stall can make useful contributions by proper care in storage and disposal of all

domestic, kitchen and shop refuse and garbage of all sorts. Such offensive materials should be stored in properly closed receptacles. The lids should fit tightly else flies will assuredly enter through the minutest chinks. Damaged bins are worse than useless for the same reason and provide admirable breeding places. Regular emptying of the contents is advised, preferably daily to arrest fermentation.

Municipal authorities should provide efficient street containers and under effective supervision arrangements should be made for the speedy removal of the refuse. In the tropics fermentation sets in very easily and once it has started, it proceeds very rapidly. There is unfortunately a lamentable defect in the civic life of India in this respect. Similarly owners of horses and cattle should exercise strict vigilance over stables and yards. The floors and drains should be kept in good conditions and thoroughly clean. Further the household refuse or refuse from stables, cattle-houses, poultry sheds etc., should under no circumstances be allowed to be dumped within a distance of half-a-mile from the nearest habitation. Such refuse should preferably be buried, otherwise burned.

Chemical control.

Horse and cattle dung when required for manuring need special treatment. For this reason chemicals, such as chloride of lime, will destroy the valuable properties of the manure. Further, the mixing of chemicals with manure requires very careful supervision if the destruction of fly larvae be the object.

Biothermic method.

By this method fly larvae can be destroyed when developing in stable manure. This is based on the following principle. When the manure is compressed by close packing, the centre of the heap becomes so hot owing to fermentation as to be quite sufficient to kill all fly larvae which have hatched out from eggs previously deposited by flies in the fresh manure. Quite a considerable number of larvae escape the heat in the centre by migrating to the outer and cooler parts of the heap. Many of the larvae which thus survive will when mature, endeavour to reach the cool, dry earth in which to pupate. Their migrations are prevented by an open channel constructed all around the manure heap in which they are trapped and destroyed in cresol solution. It is also necessary to rake off the outer exposed surfaces and turn them into the hot centre of the heap. A large number of larvae may also be trapped if the manure is stored on racks over water.

The above method of controlling fly breeding applies only to horse-manure and not to cow-manure. The temperature does not rise to the same extent in the latter as in the former.

Other methods.

Remains from slaughter houses should be removed as quickly as possible. They should preferably be buried. Incinerators are no doubt very useful in disposing street refuse but they require very careful supervision, and if there is any neglect in this respect, such places are often fruitful sources of fly breeding which takes place on an extensive scale. As garbage is sometimes required for reclaiming waste and low lands, it should be profitably utilised as far as possible.

In Norway pig manure which is a favourite nidus for house-flies can be made

innocuous if covered with a layer of cow dung. In the same way in India cow-dung may be covered with a layer of earth to make it less attractive to flies.

Disposal of human excreta.

The disposal of human excreta requires special attention because of its relation to epidemic diseases and its particular attraction to flies for breeding. The problem arises in a most pressing form in rural areas where of course no water-borne system exists. The construction of a sanitary latrine solves many problems.

Bore-hole latrine.

Of these the well-known bore-hole type of latrine has been found to be most suitable. It consists in principle of boring at least 20 feet in depth and 14 inches wide. It is covered by a suitable squatting board. The contents are in a liquid condition and if a kerosene film is used, it will prevent fly breeding. This type of latrine is economical and easy to construct, requires little supervision, is hygienic, prevents fly-breeding and produces little or no offensive odour. If used by five or six persons it will last for over 6 months.

Trenching.

Where the system of collecting and removing night-soil is employed, large quantities may be buried in trenches 4 ft. deep and 3--3½ ft. wide and with vertical sides. The surface must be effectively sealed with earth, grass or leaves and any blow-holes formed due to escape of air and gases should be sealed up.

Composting.

Indore process: This was originally based upon the natural process of conversion of the waste products of agriculture whereby the residues of plant and animal life are converted into humus through the agency of fungi and bacteria (Howard and Wad, 1931). It was later adapted as a simple solution for the sanitary disposal of the night soil and rubbish and other organic material which compose the ordinary street refuse (Mieldazis, 1934). The temperature reached is 160°F, and this high temperature is generally maintained in the compost heaps for at least three weeks; this serves to kill not only all pathogenic bacteria and helminth eggs but also fly maggots living in the cooler parts especially at the surface for which the compost has to be turned. Smell is also reduced to a minimum.

The Calcutta system is a modification of the Indore system in which the compost is made in brick lined pits instead of in trenches.

Calcutta system: (1) A battery of brick lined trenches 12 ft. long, 4 ft. deep are constructed. Channels formed of loose bricks, are so laid beneath the trenches, as to provide amply for drainage and aeration.

(2) An appropriate amount of refuse is dumped daily into successive trenches, one trench may if necessary be used for 2 days' supply of refuse. The refuse is sorted; bottles, tins and other incombustible materials are taken out and put on one side for subsequent disposal.

(3) The sorted refuse is spread loosely over the surface of the trench. A layer of about 6 to 10 inches is required. The refuse is drawn up towards the sides so as to form a hollow into which the night soil is to be dumped.

(4) Crude night soil (undiluted with water) is poured direct from night soil pails on the layer of refuse.

(5) Immediately after adding the night soil, the refuse is thoroughly mixed with a rake. The cooly stands on the edge of the trench. The mixture of refuse and the night soil is then drawn into a heap at one end of the trench where it is left undisturbed for a week. The heap is not watered. In very wet weather it may be necessary to protect the heap from excessive moisture.

(6) At the end of a week (during which 7 other trenches will usually have been similarly filled) the rubbish is turned and is drawn over to the other end of the trench where it is left to mature for 2 weeks. It is then removed and stacked in a heap on a concrete floor preferably under cover for a further 2 weeks by which time it is ready for use.

Thus at the end of the 5th week a continuous daily supply of humus becomes available for agriculture. The whole process, when properly carried out is free from fly breeding. Maggots may occasionally be observed at the surface of the heap, but these are killed after the first turn. The trenches are constructed as to prevent the escape of maggots. The average temperature recorded during the first two weeks is 140°F. At the end of this period the temperature gradually falls to normal. The occurrence of smell except at the time of dumping night soil, is no more than that which is normally associated with an efficient septic tank.

Traps, baits, fly papers, sprays etc.

It is no doubt true that a very large number of flies may be caught in properly baited traps but experience shows that although they may be useful adjuncts to other ways of controlling flies, they are, however, not very satisfactory.

Vinegar in itself is an excellent bait for the fly trap but the addition of sugar increases its attractiveness. Formalin (40%) is not constantly attractive to flies but it makes an excellent fly poison when combined with beer or milk. Sweet milk does not possess any advantage over sour milk but combined with bread, sweet milk is very attractive, but not so much so as formalin or alcohol mixtures mixed with bread. Beer is a very attractive bait under certain conditions, fresh beer being more so than stale beer; it combines readily with formalin but not with alcohol. Overripe bananas are superior to ordinary ones. Slightly decomposed fish is more attractive than fresh fish. Cane sugar and syrup have relatively low attractive values when used alone. The value of sticky fly paper is materially increased by placing small bits of attractive baits such as banana in the centre of the sheet.

To get the best effect from formaldehyde as a fly poison it will be necessary to add bait, especially sugar, to counteract the deterrent effect, sugar being the most attractive and permanent bait. The formula to be employed is:

40% formaldehyde	1 fluid ounce.
Filtered lime water	$\frac{1}{2}$ pint.
Sugar	$\frac{1}{2}$ ounce.
Water	add to make 1 pint.



A simple form of trap may be devised as follows: The poison is placed in a bottle, and the mouth is closed by means of a platform of absorbent material from the centre of which a stem of the same material passes down into the fluid. The top is wetted with the fluid at the commencement and is kept wet by capillary attraction. Such an arrangement may easily be made by blotting paper. Lloyd (1920) found 5% formaldehyde as effective as 1% sodium fluoride.

The common blue-bottle, *C. megacephala*, is particularly attracted to ripe fruits especially mango and jack fruit; country toddy is a very suitable bait for these flies on which they feed voraciously and are intoxicated and killed.

Sodium arsenite (commercial) is an excellent fly poison but dangerous to use in the household. It may be used in the following way: $\frac{1}{2}$ lb. of arsenite, $2\frac{1}{2}$ lb. of gur (treacle) and $2\frac{1}{2}$ gallons of water.

A large majority of fly-spraying fluids can knock down and stupefy but do not kill all the flies. They are of little use in the open especially in warm climates. The use of fly sprays is therefore limited to hospitals, private houses, hotels etc. An emulsion of pyrethrum extract and kerosene is a moderately effective fly spray.

Fly paper.

Household fly papers may be made as follows: Resin 12 oz., Ground-nut oil 5 oz., Crude vaseline 1 oz. The resin should be reduced to powder and the ingredients heated together, without boiling, until all the resin is dissolved. Strips of paper, tape, string or wire dipped in this preparation may all be used with advantage. If systematically used, it will kill a very large number of flies.

Dissection of fly larva.

After killing the fly larva in hot water, fix it in water on paraffin block by means of two pins placed at both ends of the body. Pinch off the skin from the dorsal surface with a pair of forceps and make an incision with scissors. Carry the incision forwards and backwards and reflect the cut ends of the skin laterally. Remove the fat body from the body cavity. The characteristic feature about the internal anatomy is the enormously enlarged salivary glands.

Dissection of adult fly.

Remove wings and legs. Snip off the coxae with a pair of scissors. Hold the fly gently between the index finger and the thumb; with a knife-edged needle or preferably a safety razor blade make a small incision in the centre of the abdomen and carry it well forwards up to where the head joins the neck and backwards up to the anterior part of the last segment. The waist or the constricted 1st abdominal tergite must be carefully incised; this always gives some trouble. The thorax on either side is incised longitudinally. The incision is carried deep into the muscle and continued both forwards and backwards.

Pin the fly on a dissecting paraffin board in 4% formalin solution and remove the cuticle together with muscles from the abdominal and thoracic segments. The entire alimentary system stretching from the head to the last abdominal segment will come to view.

When only the reproductive system has to be exposed, dissection may be confined to the abdomen only.

Preservation of flies.

Flies should be pinned while still in a fresh condition with the muscles and tissues pliable. The pin should not be passed through the fly on the median line since it may destroy the acrostichal bristles on both sides. It should be passed through the post-scutellum to one side of the middle line. The hypopygium of both sexes should be exerted and fully extended. This is particularly important in the case of male flies. The legs should be drawn away from the abdomen, *i.e.*, they should be fully extended.

Preservation of larvae.

Larvae should be killed in hot water and preserved in 80% alcohol. 4% formalin is an unsuitable preservative in the case of fly larvae.

Catching.

Flies should be caught in fly nets. A fly net is a bamboo ring set in a wooden handle about three feet long. To the ring is fixed a cone-shaped mosquito netting.

Breeding flies in the laboratory.

A very large number of methods have been described, which though they are efficient, yet they are not suitable for application in this country. Roy and Siddons (1940) have suggested the following method for breeding some common flies which may be used for experimental work.

Wild flies of the desired species are caught in food-stalls, bazaars, slaughter-houses, meat-stalls, etc. Sometimes they are only available on carcasses. As the females are alone required, they should be separated from the males. The eyes are commonly approximated in male flies but this condition is not a reliable guide unless the worker is familiar with both sexes of the species. For sex determination it is necessary to examine the last visible segment. In females it is simple in contour, tapering towards the anus. Moreover, females caught in the wild state will usually be found to be gravid, the abdomen distended with eggs and showing opaque white ventrally. In males the last segment is clearly modified to form part of the massive and complex genitalia and it is frequently of a different colour from the rest of the abdomen.

Gravid females are selected and confined individually in ordinary lamp chimneys, the tops of which are closed by gauze or mosquito-netting stuck down with plasticine. A thoroughly wet, but not dripping, wad of cotton-wool is placed on top, allowing communication with the outside air. This is the source of the fly's water-supply and must be replenished at least twice a day during the hot season. The chimney is placed upright in a close-fitting enamel dish containing the appropriate nidus (see table). Carbohydrate must be provided for food, preferably as sugar or 'gur', either under the cotton-wool or in the receptacle containing the nidus. A small quantity of the nidus, *e.g.*, horse manure, if *Musca vicina* is being cultured, is placed in the pan and when the eggs have been deposited, this is transferred to a larger quantity of the manure contained in another steep-sided pan or dish, about 12 inches in diameter, provided with a layer of sand at the bottom, half an inch in depth.

No reliability can be attached to the absence of contamination by flies in horse-dung supposed to have been collected soon after it has been passed.

Heating the manure is therefore essential in order to ensure it being free from other eggs and larvae. It should be properly broken up and mixed with a little water and heated in a closed receptacle just short of the boiling point. It is then allowed to cool, care being taken not to remove the lid and thereby expose the manure to air.

Species of fly.	Egg-laying medium.	Food.
1. <i>Chrysomyia rufifacies</i>	Meat	Meat-juice, sugar and water.
2. <i>C. megacephala</i>	"	
3. <i>Lucilia cuprina</i>	"	
4. <i>Synthesiomyia nudiseta</i>	"	
5. <i>Sarcophaga dux</i>	"	
6. <i>S. ruficornis</i>	"	
7. <i>Musca nebulosa</i>	Horse-dung	
8. <i>M. vicina</i>	"	
9. <i>M. yerburyi</i>	"	
10. <i>M. sorbens</i>	Stool	
11. <i>M. vetustissima</i>	"	

When the nidus is meat, it should not be boiled, as fly larvae do not thrive on boiled meat; slightly putrid meat is preferable. Care must be taken to see that the meat is not already contaminated by the eggs or maggots of other flies. Flesh-flies in particular may deposit their maggots through the meshes of the gauze and hence closed glass-topped cages are better for rearing these flies. Small sized mosquito-feeding cages (6" x 9" x 6") are very useful for this purpose.

When the eggs (or maggots in the case of *Sarcophaga*) have been deposited, the whole is transferred to a larger quantity of the same medium contained in another steep-sided dish, about 12 inches in diameter, provided with a layer of dry sand for pupation, and the dish, suitably labelled, is kept in a fly-proof box or cage. The pupae are later removed to a separate cage for breeding out the adults. The pupae of *Synthesiomyia nudiseta*, which is known to cause secondary myiasis, are enclosed in cocoons which may be completely invested with sand grains and hence missed.

Flies mate readily in captivity, and as the sex ratio is roughly 1:1, except in *Chrysomyia rufifacies* Macq., there should be no difficulty in breeding them. In the case of *Chrysomyia rufifacies* it will be necessary to breed from several wild females, as our experience shows that the progeny of a single female are all of the same sex (Roy and Siddons, 1939).

During the summer season in Calcutta when conditions are most favourable for fly-breeding, flies will oviposit four to six days after emergence and the second complement of eggs will mature in the same period after the first batch has been deposited. During the winter when the atmosphere is comparatively colder and drier, this period is considerably extended even up to a week, and the period of development from egg to adult is also prolonged. In places in India where

the winter is more severe, it may be necessary to place the breeding cage where it can be artificially heated to 70-80°F. and plenty of moisture should be provided.

It must be pointed out that the essential points in the details of any method connected with the continuous breeding of flies are:—

- (1) Hydrolysed protein diet for nutrition of the ova.
- (2) Avoidance of excessive or too little moisture in the culture medium.
- (3) Presence of abundant air which is essential for the growth of the larvae ; for this purpose the horse manure should be lightly placed in the pot and must not be pressed down with the finger.
- (4) Abundance of food in the medium.

One other precaution is necessary ; the protection of the pupae from the visitations of parasitic *Hymenoptera*. A small species (*Spalangia sp.*) may gain access through the meshes of the wire gauze of the fly-proof box and parasitize pupae of *Musca spp.*

ACALYPTRATA MUSCOIDEA

Acalyptrata Muscoidea, which are generally of a small size, are divided into many families ; among them the following are important.

(a) Drosophilidae (fruit-flies), (b) Sepsidae (flies with constricted waist), (c) Phoridae, and (d) Chloropidae. All are small and breed either in putrid animal or vegetable matters.



Fig. 95
Pupa of *Drosophila melanogaster*.

Family Drosophilidae.

They are minute yellowish flies and often swarm over ripe fruits. The eggs are deposited in the same situation. The pupae which resemble minute seeds are sometimes encountered in human faeces and can be easily recognised by the long tubular spiracles.

D. melanogaster Meig. Abdomen shining black ; each segment with a basal yellowish band ; mesonotum and scutellum yellowish red ; on the inner dorsal surface of the basal joint of the front tarsi are short, stiff, black bristles which are confined to one diagonal row.

Family Sepsidae.

They are small black flies and are characterised by marked constriction in the waist. They breed in decaying animal and vegetable matters. *Piophilina casei* breed in cheese and may occasionally find their way into human intestine.

Family Phoridae.

They are small or minute with a hunched-back appearance. Antennae of peculiar form ; the third joint large and concealing the others, spheroid with an apical arista. The wings have two heavy veins anteriorly which reach only half way to the apex of the wing, and three or four much lighter ones which run obliquely across the disc of the wing.

In the genus *Apiochaeta* spurs are present on four hind legs.

A. scalaris Mg. (*A. ferruginea* Brunetti). It is extremely common all over the

plains of India, and has been recorded from most of the tropical and subtropical regions of the world.

This species is of remarkable interest as it is thought to be capable of passing its entire life-cycle in the human colon.

The fly is of extremely small size and the larva is also proportionately small. The adult is of an ochraceous or brownish yellow colour. The frons is furnished with 4 rows of 4 macrochaetae each. Tibiae with a distinct row of short bristles on outer side; the four posterior tibiae with two terminal spurs, the hind pair having a close row of very minute hairs on the outer side adjacent to the row of bristles.

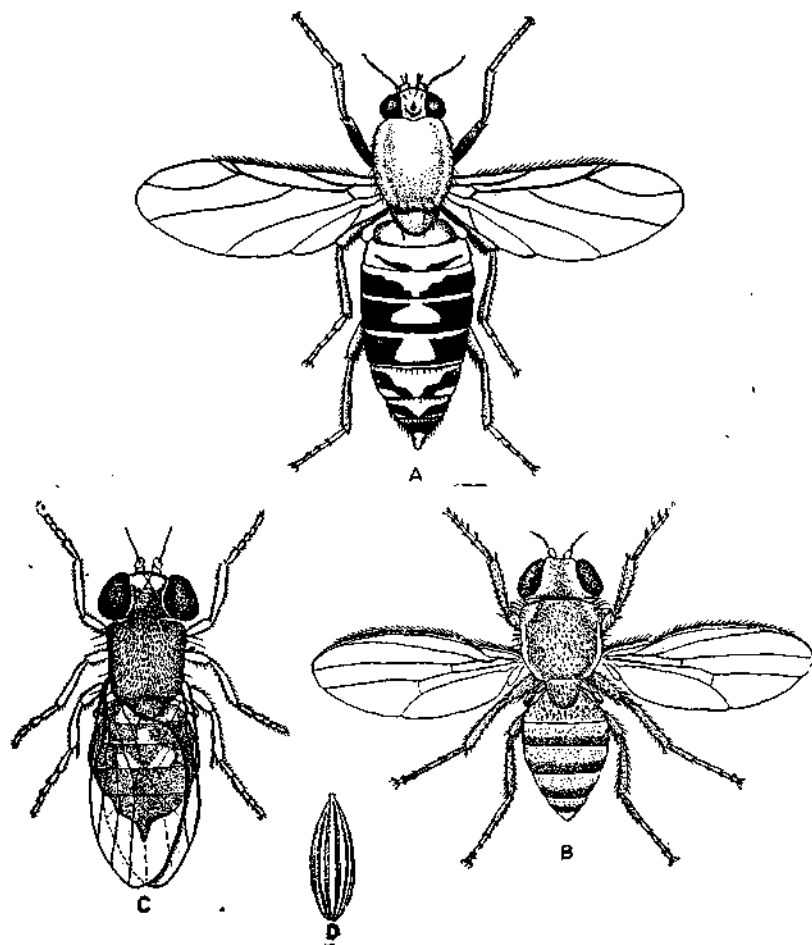


Fig. 96

- A. Female *Apiocnata scalaris*.
 B. *Drosophila melanogaster* (the fruit-fly).
 C & D. Adult and egg of *Hippelates pusio* (After Hall).

The larva is extremely small and the pupa looks like a small seed. The posterior larval stigmata are seen in the form of two small brownish papillae situated close together.

It takes about 12 to 15 days for the first generation to emerge. Pupation takes place from 5 to 8 days after oviposition and the emergence of the adult occurs seven days later.

A. scalaris is a common fly breeding in human faeces. It also breeds in the excrement of laboratory animals, both fresh and putrid meat, and on small dead animals, e.g., lizard, snail, caterpillar etc.

Family Chloropidae (Oscinidae).

They are popularly known as eye-flies on account of their habit of hovering over the eyes. They are black in colour and of extremely minute size. They are all directly related with the carriage of the causative agents of conjunctivitis, which, when it is fly-borne, appears in an epidemic form and mostly affects babies and children. The fly merely acts as a mechanical carrier. These flies have also been incriminated with the dissemination of yaws. The spirochaete of yaws can remain alive in the digestive tracts of *Hippelates pallipes* Loew.; they retain their motility for a much longer period in the oesophageal diverticulum than in the stomach. In the stomach the motility is lost very rapidly. The method of infection, therefore, has been suggested by Kumm *et al.* (1935) to be by the vomit drop.

In this family both male and female vertex are dichoptic; arista is bare or finely feathered which can be detected under the microscope; the third antennal segment is globular; males can be recognised by their terminalia.

Hippelates pusio Loew (*H. flavipes*). This is found in various places in the United States and the West Indies and acts as a true eye-fly. It does not feed on ulcers. Herms (1926) gave an account of conjunctivitis produced by this fly. Eggs have been found on decaying animal excrement, decaying fruits and vegetable matter. The life of a larva generally lasts for one week, and that of the pupa for 10 days.

H. pallipes, though more common than *H. pusio*, prefers to feed on ulcers on the feet and lower extremities. The name eye-fly does not apply so well to *H. pallipes* as to *H. pusio*. *H. pallipes* are probably of more importance as far as yaws is concerned than the other species because of their positive tropism towards ulcers on men and animals rather than towards decaying dead animal matter. This fly when feeding on yaws lesions will crawl underneath the dry scab if necessary to get fresh sero-purulent material.

Oscinis pallipes Lamb. is the eye-fly of Egypt. Antennae are reddish, blackened on the end of the 3rd joint; the legs are wholly pale.

They can be easily bred on decaying vegetable matter and horse and cow dung.

Genus *Siphunculina* (*Siphonella*).

Siphunculina funicola de Meij. is the eye-fly of India, Ceylon and Java. These flies are extremely small or minute in size. The arista is microscopically feathered. The wings are shiny. The tibiae and the tarsi of the front pair of legs and the tarsi only in the mid and hind pairs are golden. The knee joints are golden in the mid and hind pairs of legs.

Though they are widely distributed in this country, they are great pests in

places where the climate is warm and moist. They are therefore particularly common in the eastern and southern parts of the Peninsula. They are responsible for the spread of the severe form of ophthalmia found in this country especially in tea gardens and it usually occurs in an epidemic form in Assam. The fly is a serious pest from May to June. It is very active on a hot sunny day but its activity ceases when the air is cool or the sky cloudy. In the evening it settles on strings, thatch etc., hanging from the roof. It is very common in cowsheds, but only a few may be found in open fields. It feeds on warm human excretions, on the exudates of ulcers of man and animals, putrid sloughs of ulcers, the discharge from the nostrils or eyes and on cow and horse dung, either fresh or decomposed.

The proboscis in *S. funicola* has undergone a considerable modification from the normal Muscid form though it is completely retractile. There are no teeth or interdental armature of the biting *Musca*. The rostrum is reduced. There is a great reduction in the number of pseudotracheal channels. Some of the pseudotracheal rings are enormously elongated into spines. According to Senior White (1923) the fly uses the projecting free ends of the pseudotracheal rings as scarifying organs to remove dried crusts and reach the serous exudations beneath them. Roy (1928), however, found that it settles on the healthy skin at the margin of an ulcer and tries to force its proboscis through a rent in the scab to get at the exudation below.

Its natural breeding places are: (1) decomposing animal and vegetable matter (Hutson, 1928); (2) earth sodden with dung and urine (Roy, 1928); (3) ammoniacal soil (Syddiq, 1938); and (4) house thatch from the roofs of dwellings (Hamilton, 1939). Females lay eggs in batches of 5-10; the duration of the life cycle under favourable conditions of climate is about 10 days according to Syddiq and 3 weeks according to Hamilton.

Pyrethrum spray is the only remedy that can be suggested to cope with the nuisance. The adult flies are extremely susceptible to pyrethrum and there is always a great reduction in their number after spraying. (Viswanathan, 1941).

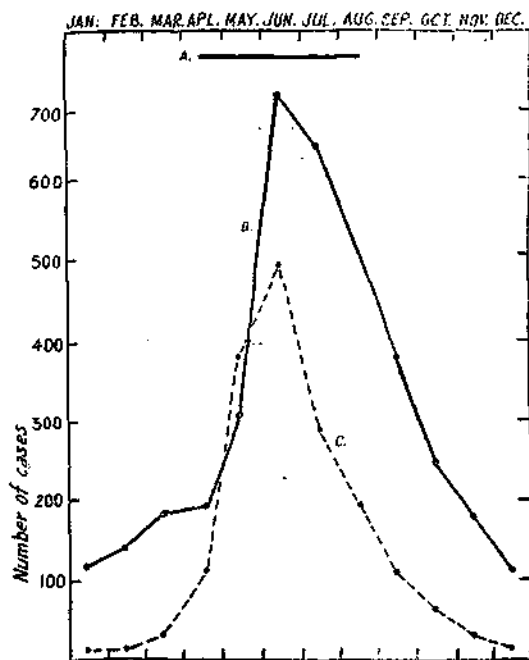


Fig. 97

Chart showing the incidence of Naga Sore (B), and epidemic conjunctivitis (C) in a tea estate in Assam during 1924-25. The horizontal line above (A) shows the period during which *Siphunculina funicola* is a serious pest.

Redrawn by Graham-Smith from the chart given by Roy.

CALYPTRATA MUSCOIDEA

Families		1. Non-biting	Genera
		2. Biting	
Muscidæ			<i>Musca</i> <i>Stomoxys</i> <i>Glossina</i>
Calliphoridae			
Subfamily	Calliphorinae		<i>Lucilia</i> / <i>Chrysomya</i> <i>Cochliomyia</i> <i>Calliphora</i>
			also { <i>Auchmeromyia</i> <i>Cordylobia</i>
	"	Sarcophaginae	<i>Sarcophaga</i> <i>Wohlfahrtia</i> <i>Æstrus</i> <i>Hypoderma</i> <i>Gasterophilus</i>
Æstridæ			
Tachinidæ			
Anthomyidæ			<i>Fannia</i>

Study of the external characters of a fly.

Such studies are necessary for the determination of the genus and the species. Principally dry mounted specimens are used. Slide preparations especially of the hypopygium may also be required. It must be clearly realised that except in the case of the common house-frequenting flies specific identifications are difficult and it is desirable that the specimens should always be sent to an expert. No reliance can ever be placed on identifications made by non-experts. Ordinarily one should be satisfied with the genus which is not so difficult.

For the identification of flies certain terminology which are commonly used must be understood. The important ones are:—

- (a) Acrostichal and dorsocentral bristles on mesonotum ;
- (b) Eye length which is the longest axis of the eye viewed from the front ;
- (c) Parafrontalia—these are the frontal portions of the genæ from vertex to bases of antennæ ;
- (d) Vertex: the posterior edge of front ;
- (e) Hypopleura: form the lateral walls of metathorax and lie just below the posterior spiracles ;
- (f) Jowls: portion of genæ between eyes and the lower edge of the cheek ;
- (g) Humeral callus: a plate lying just above the propleura ;
- (h) Genæ: below the region lying between the eyes and the bases of the antennæ.

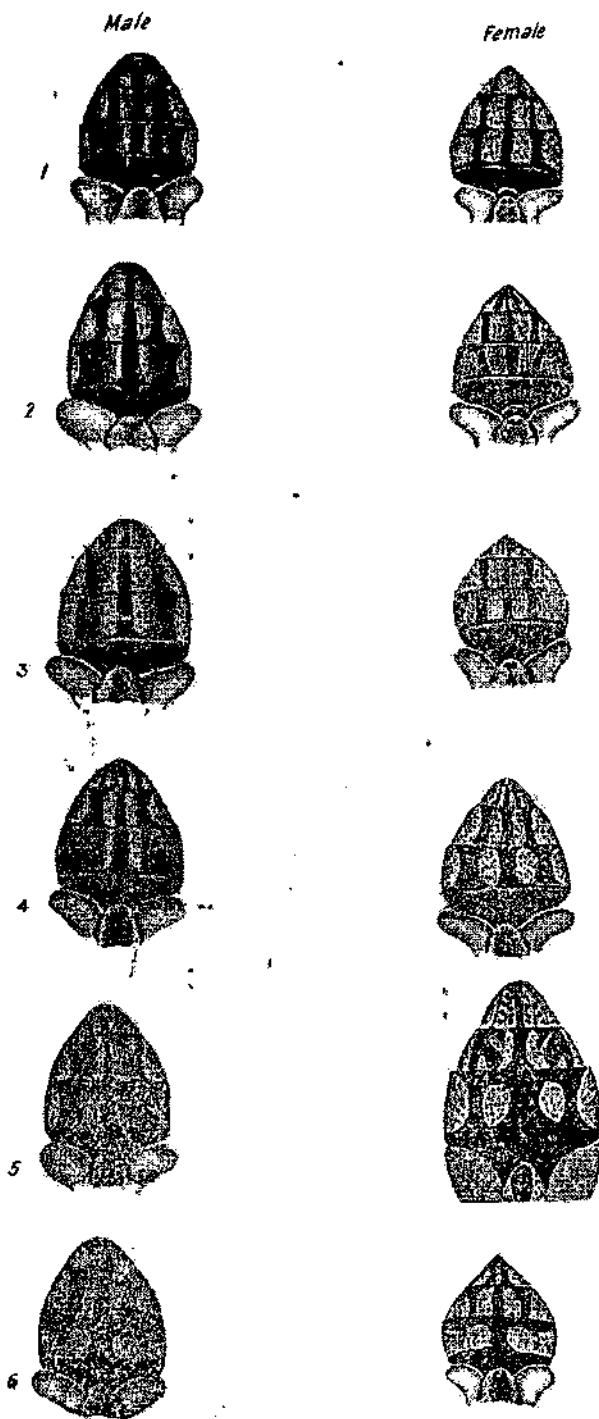


PLATE IX.

Dorsum of abdomen of some common house-frequenting and camp flies of India. (After Patton and Senior White, 1924.)

1. *Musca domestica*, male and female

4. *Musca yerburyi*, male and female.



PLATE X.
Rattus norvegicus.

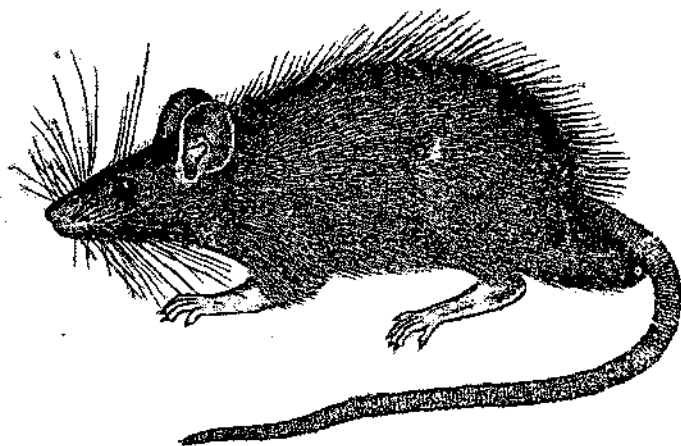


PLATE XI.
Rattus rattus

The Calyptrata Muscoidea includes the house-fly and other flies which are known to play significant roles in the carriage of pathogenic organisms. This group may be divided into a number of families as follows:

Families of Calyptrata Muscoidea.

(1) Muscidae. Hypopleura without any tuft of bristles; 1st posterior cell narrowed to any degree, in fact it may be almost closed; arista feathered to the tip either on both sides or on one surface only. The genus *Musca*, the members of which are mostly non-biting, is included in this family. These flies are mostly oviparous though a few are larviparous and the arista is feathered on both sides to the tip. Among the biting Muscidae there are three important genera, *Stomoxys* or the stable fly, *Glossina* or tsetse fly, and *Lyperosia* or horn fly.

(2) Calliphoridae. Hypopleura with a tuft of bristles; 1st posterior cell as in Muscidae:

This may be divided into two subfamilies, Calliphorinae and Sarcophaginae. The former flies are all metallic coloured, either blue or green. The arista is feathered on both surfaces along its entire length. This subfamily contains 4 genera (a) *Lucilia*; (b) *Chrysomyia*; (c) *Cochliomyia*; and (d) *Calliphora*. All are oviparous.

Sarcophaginae are grey coloured flies with striped thorax and chequered abdomen. It contains 2 genera, (1) *Sarcophaga*; and (2) *Wohlfahrtia*. These are all flesh-flies and are larviparous; they have retractile proboscis. In *Sarcophaga* the arista is plumose up to the middle, whereas in *Wohlfahrtia* the arista is bare.

(3) Anthomyiidae: They are not so important as the other two families. The larvae are parasites of plants especially in the garden and many of them parasitise other insect larvae chiefly of Lepidoptera; one genus *Fannia* sometimes visits dwelling houses.

The adult flies closely resemble house-flies from which they can be easily distinguished by the course of the 4th longitudinal vein which is more or less straight, the first posterior cell being thus widely open.

(4) Oestridae. Members of this family lead strictly parasitic lives and infest cattle, horse, and other animals. In the adult stage the mouth parts are entirely suppressed.

(5) Tachinidae. These are of great practical importance as they parasitise insect larvae, chiefly of Lepidoptera. The body and legs are covered with stiff hairs or bristles.

FAMILY MUSCIDAE.

Genus *Musca*:

The following are the main features of the genus *Musca*. (1) Generally small or moderate in size. (2) Greyish, blackish—or bluish grey integument without any metallic lustre. (3) The arista is feathered on both sides up to the tip. (4) The proboscis is retractile. (5) The 4th longitudinal vein bends forwards at a sharp angle, so as almost to close the first posterior cell. (6) The sternopleural bristles are arranged as 1:2.

Patton has classified the genus *Musca* into three groups in accordance with their feeding habits. Group I includes the true house and bazaar-frequenting flies and though at times may be found on animals and show a haematophagous tendency, they are mainly associated with man, his dwellings and his food. They breed in garbage and excrement of all kinds. This group includes *M. domestica*, (*pumila* Macq.) and *M. yerburyi* Patton. Flies in group II are non-biting, haematophagous species and are only found on animals and in their vicinity and breed almost exclusively in cow-dung. These include *M. ventrosa* Wied., *M. craggi* Patt., and others. Group III consists of the true biting species which are only found on animals and which breed exclusively in cow-dung. These are among others, *M. inferior* Stein, and *M. crassirostris* Stein.

Medical and public health workers are primarily concerned with Group I whereas the veterinarians should be familiar with those that bite cattle and are responsible for the deterioration of the health of these animals.

The research worker may feel the necessity of identifying the common house-frequenting flies and in this respect a key does not seem to be very helpful. It is for this reason that a more detailed description taken mainly from Patton has been given. It may be pointed out that the typical *Musca domestica*, the house-fly of temperate climates, does not occur in India and it is doubtful if it is found in Africa. Both are found in China. Its place in the tropics has been taken by *M. vicina*. The latter fly no doubt bears a close resemblance to *domestica*, the only difference being in the male which has an exceptionally wider frons in *domestica* than in *vicina* in which the frons is very narrow; in fact the two eyes nearly touch each other. It may be of interest to know that the *vicina* found in the hills above 5,000 ft. elevation is intermediate between the true *domestica* and *vicina* and interbreeding experiments between the hill and plain varieties of this species show a tendency for the frons to become narrow.

M. domestica L. The vital importance of horse-manure and pig-dung to the development of this species has been fully realised. There is no essential difference between horse-manure and pig-dung as regards their power to attract the ovipositing flies. Pig-dung is highly favoured as a medium for egg-laying in many parts of Europe but when this is covered with cow-dung, the oviposition is stopped.

Musca vicina (*Musca domestica vicina* Macq.). It is the commonest house-frequenting fly in the tropics. It breeds in domestic, stable and garden refuse, horse manure, night soil in patches, but more extensively in trenches, and occasionally in piled up cowdung mixed with organic and vegetable refuse matters especially during the rainy season. In fact it will breed in any fermenting material, but if covered with a thin layer of earth or cowdung, it will at once fail to attract the ovipositing flies. All house-flies are oviparous.

In both sexes of *M. vicina* the mesonotum has four well defined dark stripes. In the male the abdominal terga have a median broad black stripe; the apparent first segment with a broad black band; tergum 3 on either side of the median band orange which is bordered on both sides by a silvery stripe; tergum 4 greyish yellow.

In females the abdominal characters are almost the same as in males except

that terga 1, 2 and 3 are more extensively orange ; apparent first segment black or dark brown anteriorly.

Musca nebulo F. Roughly it comprises about 5% of the catches made from dwelling houses though in certain houses it may be entirely absent, all being *M. vicina*.

It has the same breeding habits as *vicina*. Like *vicina* both sexes possess 4 thoracic stripes. In male flies the median abdominal stripe expands anteriorly on the first apparent segment to form a dark band across the anterior half but it is narrower than in *vicina* ; tergum 3, in addition to a silvery stripe, has silvery patches at the margins ; on tergum 4 silvery stripe and silvery patches are more prominent.

The female vertex and cheeks are creamy white ; abdomen is light orange ; silvery stripes and spots on remaining segments well marked.

M. yerburyi Patt. It is not a house-frequenting fly but is essentially bazaar fly though found in considerably smaller numbers than *vicina* and *nebulo*. It breeds in entrails of animals, decomposed fish etc. Eggs are also laid in animal excrement.

In both sexes there are four mesonotal stripes. In the male terga 1 and 2 yellow ; tergum 3 orange with a broad median black stripe bordered with narrow to broad silvery stripes but without silvery marginal spots.

In the female terga 1 and 2 are the same ; tergum 3 with a broad, black median stripe bordered with broad silvery stripes, also well marked silvery marginal spots.

M. sorbens Wied. Eggs are laid principally in large accumulations of cow-dung. At times the larvae are found in isolated patches of cowdung and small deposits of human faeces.

M. vetustissima (*M. pumila* Macq.). It is essentially a bazaar fly and is occasionally found in camps. It breeds in entrails of animals and fish and sometimes eggs are laid in animal excrement.

Both *sorbens* and *vetustissima* are characterised by the presence of two stripes on the thorax. In male *sorbens* the eyes are well separated ; in *vetustissima* they meet in the middle line. Other characters by which the male of the two species can be distinguished are: in *sorbens* the thorax is grey ; abdomen orange ; apparent first segment either dark or light brown ; apparent 3rd and 4th segments with a median black stripe and admedian and marginal silvery spots. In *vetustissima* the thorax is bluish grey, abdomen dark orange ; apparent first segment black ; other 2 segments similar to those of *sorbens*.

The female *sorbens* has grey thorax with two black stripes separated before the suture ; abdomen blackish grey with black bands and stripes. The female *vetustissima* has the same thoracic stripes but the abdomen is bluish grey with black bands and stripes.

As has been stated before the true house-frequenting flies in the tropics are mainly *M. vicina* and *M. nebulo*. Where the dwelling house adjoins the cow-shed *M. sorbens* are also found. *M. vetustissima* and *M. yerburyi* are really bazaar-flies though occasionally isolated specimens may be caught in-doors.

The following two species, *M. crassirostris* and *M. inferior*, have the proboscis structurally adapted for scratching and tearing through skin and drawing blood.

M. crassirostris Stein.

It is a widely distributed species. It is larviparous and breeds in cattle dung.

It is readily recognised by its large size, olive green colour and by the large bulbous mentum.

M. inferior Stein. It is also widely distributed in the Oriental Region. It is oviparous and breeds in cattle dung. The eyes in the male are widely separated. It is a large species which is easily identified by the character of the proboscis and by the presence of hairs on squamae, being the only species which has such hairs.

FAMILY CALLIPHORIDAE.

Subfamily Calliphorinae (metallic coloured bluish or greenish flies).

Genus *Lucilia*. (Green-bottle flies).

It is characterised by setae on a considerable part of the upper and under surface of the 3rd longitudinal vein, bare squama and presence of well developed dorsocentral and acrostichal bristles on both prescutum and scutum. The arista is plumose to the tip. The sternopleural bristles are 2:1.

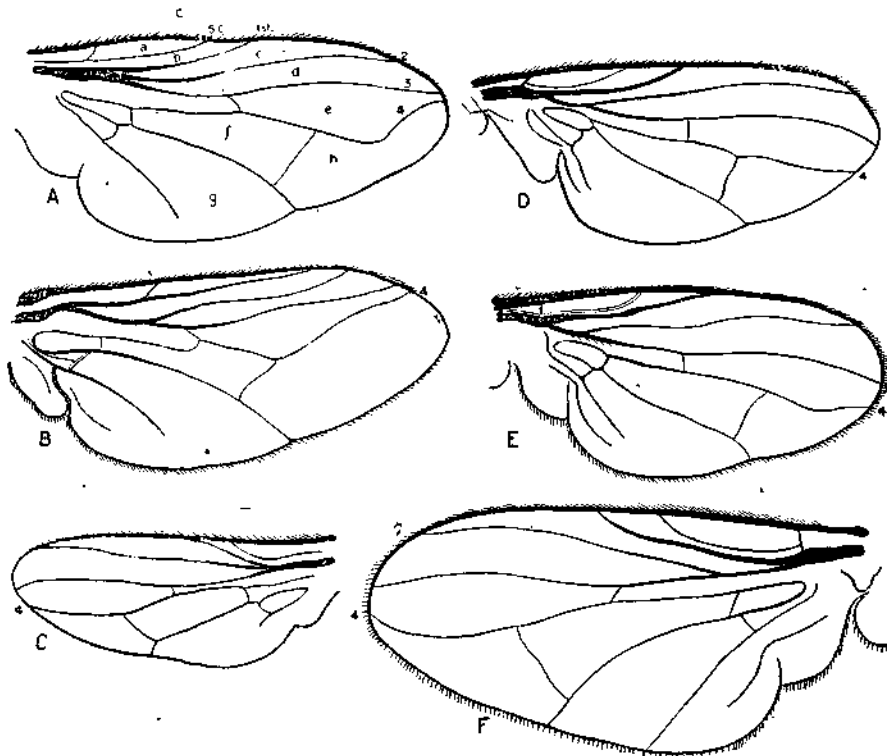


Fig. 98

Wings of (A) *Musca*, (B) *Glossina*, (C) *Lyperosia*, (D) *Fannia*, (E) *Stomoxys*, (F) *Muscina*.

c, costal vein; s.c., subcostal vein; l-4., longitudinal veins; a, costal cell; b, subcostal cell; c, marginal cell; d, submarginal cell; e, first posterior cell; f, discal cell; g, third posterior cell; h, second posterior cell.

These flies are commonly called green-bottles and all have a bluish and bright metallic colouring. The females are oviparous and larvae develop mostly in decaying animal matter. Some of these flies have the habits of blow-flies. Though *Lucilia* flies will occasionally blow meat placed outside but they seldom enter the house in search of food.

L. caesar L. Found in the Palaearctic Region. It has also been recorded from New Zealand, eastern Australia, and South Africa. The subcostal sclerite has one or more upstanding bristles. In *L. sericata* Mg. and *L. cuprina* Wied. the subcostal sclerite has fine decumbent pubescence.

L. sericata and *L. cuprina* are cosmopolitan both being common in the Oriental Region. In *sericata* the body is rounded, metallic green with sparse pollinosity on the mesonotum. In *cuprina* the body is elongate, metallic coppery with rather dense pollinosity on the back. In the female *sericata* the parafrontals have short, fine, decumbent stiff hairs among the frontal and fronto-orbital bristles; whereas the female *cuprina* possesses bare parafrontals apart from frontal and fronto-orbital bristles. In the male *cuprina* the abdominal sternites bear tufts of long hairs which are absent in the male *sericata*.

In Australia, New Zealand and Great Britain sheep are infested with larvae of *L. caesar* and *L. sericata*. The eggs are deposited in batches of 45 to 275 eggs in the soiled wool of the sheep. The wool of the hind quarters is most frequently blown by them. The maggots on hatching penetrate the skin. In this way extensive festering sores are caused.

L. cuprina which is an Ethiopian and Oriental species is widely prevalent in India; it is a common bazaar-fly; *L. sericata* is found in North Western India. Both breed in animal matter.

Genus *Chrysomyia*.

It is also like *Lucilia* a metallic coloured fly but differs from the latter in many important respects. It is bluish in colour and hence called blue-bottle. The pre-scutal and scutal acrostichal bristles are almost absent and the prescutal dorso-central bristles are practically wanting. The sternopleural bristles are commonly 1:1 and in some cases 2:1. The upper surface of the squama is hairy.

The arista is plumose to the tip and in the male the eyes are close together whereas in the female they are wide apart. They generally breed on the carcass of both large and small animals.

The spiracular slits of the larva are directed in one direction, i.e., towards the scar and the peritreme surrounds the slits on all sides.

C. megacephala, Fab. This species is very distinct on account of the much enlarged upper facets in the male and in both sexes by the black prothoracic spiracles and the dark squamae which have on the upper surface a white spot exteriorly.

Eggs are laid in (1) dead animals such as dogs, cattle, rats and big birds; (2) in human faeces after it has turned into the liquid form as a result of putrefaction; enormous numbers of their larvae are commonly noticed on the floors and also in pots of service privies when they are not cleaned regularly; (3) larvae have been recovered from isolated patches of cow-dung.

Next to *M. vicina* and *M. nebulo* it frequents dwelling houses. It is greatly attracted by mango, jackfruit and country toddy ; it is quickly overwhelmed by the latter and dies.

C. rufifacies, Macq. *C. albiceps* Wied. In nature they generally choose large birds for depositing their eggs though in the laboratory at least the former will readily oviposit on the flesh of any animal. The larvae are hairy, dark in colour and predacious, feeding on other fly larvae. Both *rufifacies* and *albiceps* have the anterior spiracles white. The latter is found in Australia.

In *albiceps* there is a strong bristle just below the prothoracic spiracle which is absent in *rufifacies*.

Its method of reproduction is unique. Mating is necessary. A female produces eggs which turn into adults of one sex only, not only in the first but also in subsequent batches. Some females therefore produce only males whereas others only females (Roy and Siddons, 1939).

Chrysomya bezziana Vill. It is widely distributed in Africa, India and also further east and is a true myiasis-producing fly. It deposits its eggs always in living tissues. In the human being the larvae are found in any of the natural orifices of the body such as nose, ear, dental socket, and on rare occasion vagina. One case of cutaneous myiasis has been observed in a pilot following extensive burns on the body. In animals the larvae are commonly found in the scrotum after castration. The flies are attracted to an evil-smelling discharge which induces them to lay eggs. Patton thinks that the larva is capable of attacking living tissue such as muscle, tendons etc., causing an extension of the ulcer. This, according to him, may end fatally. No such bad effect has, however, been noticed by us and we are convinced that the larvae feed on necrotic tissues only.

The adult fly has not yet been encountered in nature. The mature larva presents a characteristic cork-screw-like appearance due to the presence of well developed intersegmental spines. Hence it is often called screw-worm larva.

The posterior wall of the pharynx of the larva is smooth suggesting that the larva is strictly parasitic.

The adult *C. bezziana* can easily be distinguished from *C. megacephala*. The squama in *bezziana* is waxy white while in *megacephala* it is greyish. In the male *megacephala* the upper facets of the eyes are large, which is not the case in *bezziana*. The intersegmental spines and the character of the pharynx will be helpful in distinguishing their larvae. The spines are normal in *megacephala* and the posterior wall of the pharynx in this species is ridged.

Genus *Cochliomyia*.

It is restricted to the New World and is characterised by the presence of longitudinal stripes on the thorax. The palpi are short and slender. It is oviparous. *C. hominivorax* Coq. (*C. americana* of Cushing and Patton) is the screw-worm fly of the New World. It is an obligatory myiasis-producing fly (Cushing and Patton, 1933), the posterior pharyngeal wall in the larva being without any ridge. According to Aubertine and Buxton (1934) it generally chooses one of the orifices of the body especially the nose for laying eggs. These authors

believe that cases of myiasis in human being are more common than in cattle. This fly is very widely distributed in America and West Indies.

The destruction of tissues alone is not responsible for the injury to the animal and the severe effects are due either to bacteria associated with infestation or to metabolic or toxic products of the larvae formed around their activity in the wound. The excessive destruction of soft tissues sometimes seen in cases infested by larvae of *Chrysomya bezziana* Vill. in man may be similarly explained.

C. macellaria F. which is a saprophYTE and breeds in dead meat was previously confused with *C. hominivorax*. The latter causes screw-worm infestations in man and warm blooded animals in tropical and subtropical America.

Genus *Calliphora*.

Squamae with a white hind border and distinct hairs above, at least towards the middle.

Eyes of female separated; abdomen dusted; dorsocentral and acrostichal bristles both presutural and postsutural are well developed.

C. erythrocephala Mg. is the well-known blue-bottle fly. It is a large bluish-black species with a more or less whitish pollinose abdomen and black legs; palpi reddish; antennae black and jowls (part of face below cheeks) red, with black hairs.

C. erythrocephala oviposits on fresh or tainted meat, on carcasses, offal, and other animal refuse. It is a cosmopolitan species though it is essentially a hill species in India and is found only during the summer when it frequents dwelling houses.

Auchmeromyia luteola F.

It represents a special group of Calliphorinae characterised by their blood-sucking habits and intermittent parasitism of the larva.

This fly is distributed throughout the whole of the Belgian Congo where it is a common pest. This species is found in close association with man and is considered to be a specific human parasite as the larvae are blood suckers and live on the blood of man. Numerous eggs are generally laid in the loose and slightly moist soil. Their characteristic markings make them easily recognisable. The maggots are unable to climb and are entirely dependent for their existence upon the habit of the local people of sleeping on floor mats. The adults are supposed to live on blood; the mouth parts are, however, adapted for sucking only.

Cordylobia anthropophaga Grün.

The genus *Cordylobia* is peculiar to Africa and is known as Tumbu-fly. The larvae are found in dogs, goats, cats, domestic rabbits and also in man causing subcutaneous tumours. The fly deposits its eggs on the ground where there is smell of animal or human perspiration. In man tumours in connection with the presence of larva are always found on parts of the skin coming into intimate contact with the ground. In animals the larva shows a marked preference for the skin of the scrotum. The life history is incompletely known. Eggs or young larvae are laid in some cases on clothing impregnated by perspiration. Pupation takes place in the soil and occupies about a fortnight.

Metazoal Immunity.

Blacklock and Thompson (1923), working with this fly, proved experimentally that an immunity against the larvae can be acquired by both men and animals. This immunity is confined to the area of the skin into which the parasite had previously penetrated, and from this area later the immunity spreads to other areas (Blacklock and Gordon, 1927). The immunity of *Cochliomyia americana* (*macellaria*) is confined to the infested area alone (Borgstorm, 1938). Mellanby (1944) suggests that a similar immunity is developed in human beings as a result of infestation with scabies mite whereby the mite population cannot increase in subsequent infestation as it does in primary infestation.

Subfamily Sarcophaginæ (flesh-flies)

Genus *Sarcophaga*.

The *Sarcophaga* flies contain many species distributed throughout all the zoological regions of the world. These flies are generally of large size though some are quite small. They are grey in colour, the thorax is banded or striped and the abdomen is chequered or tessellated. The sexes cannot be definitely distinguished from the approximation of the eyes; this can be better done by examination of the terminal segments of the abdomen. The arista is plumose on both sides up to half its length, the terminal part being bare. All are larviparous; first-stage larvæ are deposited. Posterior spiracles of the larva lie in a deep recess whose lips may be kept closed. The spiracular slits are not directed in one direction; the peritreme is interrupted in its lower part and the scar is absent or very poorly developed.

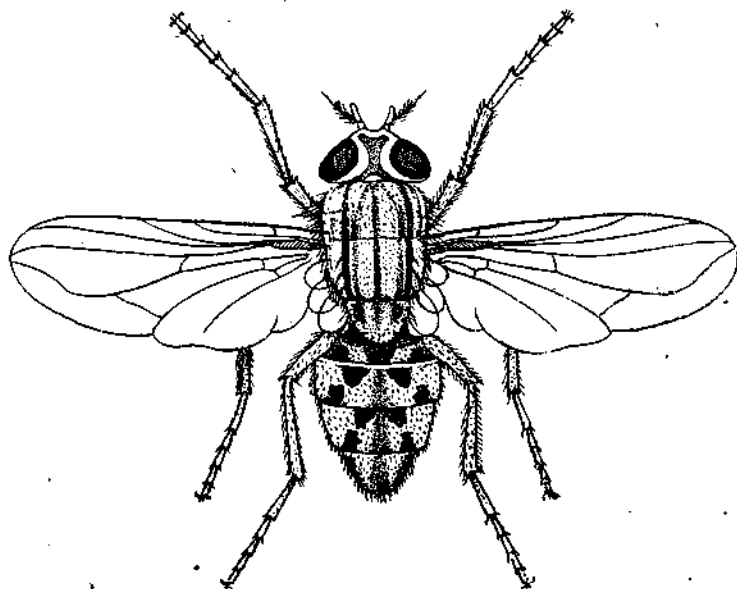


Fig. 99
Female *Sarcophaga ruficornis*.

They are called flesh-flies on account of their habit of breeding on flesh for which purpose carcasses of small animals like rats are generally chosen. Some

species also choose freshly passed human faeces for breeding. Some deposit their larvæ in human and animal tissues.

It is not really a house-frequenting fly; its visit in the house is always associated with the presence of meat. It is not necessary for this fly to sit on the pabulum for depositing its larvæ. It can drop its larvæ from a distance while flying over the meat. Ordinary wire-meshed meat covers are no protection against them. For this purpose meat has to be covered with paper.

The two common species frequently encountered in connection with cutaneous myiasis in man and animals are *S. ruficornis*, Fab. and

S. dux, Thoms. The former is by far more common than the latter. Their larvæ feed entirely on necrotic tissue and their presence in the wound may be considered as beneficial, the process of healing being greatly accelerated.

Males of both *S. dux* and *S. ruficornis* have the frontal width two-thirds that of an eye while in females the width is equal to that of an eye.

S. ruficornis Fab. Prescutellar, acrostichal bristles are either absent or only one is present. The tip of apparent fourth abdominal segment reddish. No spot on second abdominal segment. Antennæ and palpi yellow-orange. They breed on both large and small animals such as rats and big birds, also freshly passed human faeces.

S. dux Thom. Antennæ and palpi black; only prescutellar acrostichals are present. They generally breed on dead animals such as snails, snakes etc.

Genus *Wohlfahrtia*. The third joint of the antennæ is short, and the arista is not armed with bristles. The abdomen is marked with well-defined spots and there are no glistening reflections as in *Sarcophaga*; it has, however, the same breeding habits. It has not yet been recorded from India but from Central Asia, Mesopotamia, Northern Africa and Southern Russia. The larvæ of *W. magnifica* Schin. have been found in cutaneous ulcers of animals in Russia. *W. vigil* Wlk. is a frequent cause of human and animal myiasis in North America.

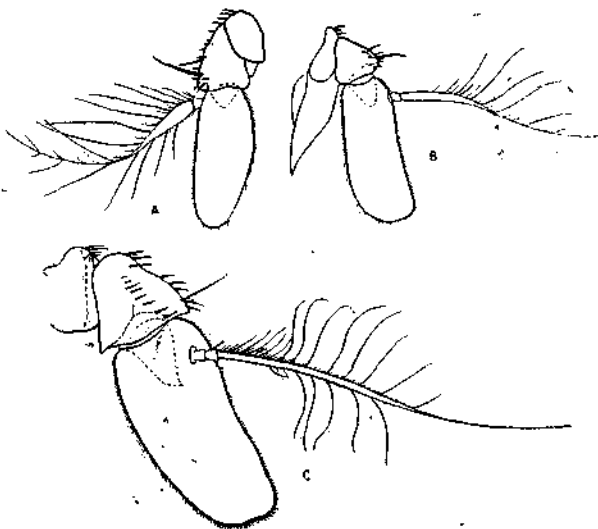


Fig. 100

- A. Arista of house-fly.
B. " " *Stomoxys*.
C. " " *Sarcophaga*.

FAMILY OESTRIDE

All members of this family lead parasitic lives and the adult flies do not take any nourishment due to the mouth being absent. They live for a short time only

for the purpose of reproduction. The following are the important genera; (a) *Oestrus* (b) *Hypoderma* (c) *Dermatobia* and (d) *Gasterophilus*.

Genus *Oestrus*.

O. ovis is primarily a pest of sheep, occasionally of goats but in some rare cases attacks man. In certain regions of the Sahara, the larvae may be deposited on the conjunctival and nasal mucous membranes of man and cause much trouble. This fly is common in the United States, India and other parts of the world but there is as yet no record of the causing of myiasis in man.

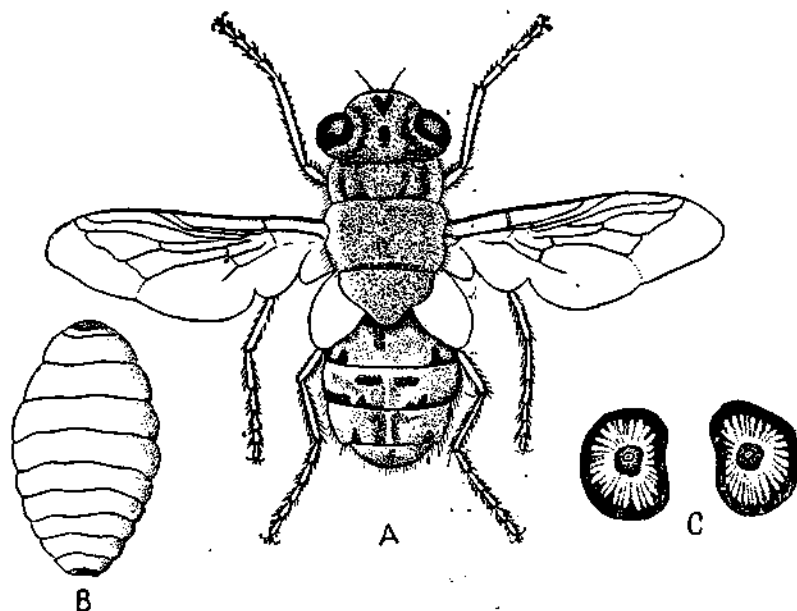


Fig. 101
Oestrus ovis

A, adult; B, larva; C, posterior spiracles.

This fly is larviparous. The larvae are deposited just inside the nose when the female fly hovers over and strikes at the nostrils. The young larvae crawl to the nasal sinuses or even to the base of the horns and attach themselves by hooks to the mucous membranes. Occasionally they may migrate to the cranial cavity or turbinate bones, where they may become imprisoned and die. The larvae usually live in the host for 8 to 10 months but may take only 6 months to mature. They crawl out and are expelled by a violent fit of sneezing. They pupate in the ground.

Genus *Hypoderma*.

All are primarily parasitic on cattle and goats. Only three species are important, (a) *H. bovis* (b) *H. lineatum* and (c) *H. crossi*. *Hypoderma* larvae enter their hosts through the skin close to the spot where the eggs have been laid. The adult flies are rarely seen elsewhere than in the vicinity of their hosts.

H. bovis DeG. is the prevalent species on cattle in Europe. The eggs are laid on the legs and more rarely on the flanks, apparently never on the back. They

are attached singly to a hair near its base. It is not known how the larvae find their way to the animal's back as the eggs are always laid on the lower parts. It is also not known where the larva assumes the second stage. The second stage larva is invariably found outside the muscular coat of the gullet and it appears that from the submucous coat of the oesophagus they pass into the latter, and then into the stomach. After boring its wall the larva eventually reaches the back of the host. It makes a small opening in the skin through which the larva escapes and falls to the ground to pupate.

The effect of the parasite, *H. bovis*, on cattle is very serious. The growth is inhibited and the production of milk is reduced.

The ox-warble fly, *H. bovis*, causes losses which have been estimated at from 2 to 7 millions sterling per annum in England alone.

H. lineatum Vill.: is the prevailing species on cattle in North America. It attaches several eggs in a series, 1—14, to a single hair.

Oviposition inflicts no pain on the animal and may be unnoticed, but if the victim becomes aware of the presence of the fly, great uneasiness and terror is excited. By their persistence in oviposition they frequently stampede herds of cattle, which take refuge in water if available.

In *H. bovis* dense yellow hairs are found on the thorax in front and the terminal hairs on the abdomen are yellow, whereas in *H. lineatum* there is uniform covering of mixed hairs on the thorax and similar abdominal hairs are orange red. The larvae can also be readily distinguished. The larvae of *H. bovis* are large and of greenish brown colour whereas those of *H. lineatum* are smaller and brownish grey. The larval life of *H. lineatum* is also much shorter.

H. bovis has not yet been recorded from India. Only *H. lineatum* has been known to occur in Northern India affecting cattle and sometimes goats.

H. crossi Patt: has been recorded by Patton from goats in the Salt Range district of the Punjab, hilly tracts of the N.W.F. Province, Baluchistan, Kashmir and Kulu valleys. The larvae are also occasionally found on cattle. The eggs are laid on the long hair and the larvae enter the skin below and remain there. There is no migratory stage as in other species of *Hypoderma*. The damage is primarily confined to hides.

It has been suggested that the injection of a few drops of paraffin or tinct. iodine into the warble holes is an effective treatment of warbles. The larva is killed and is absorbed without pain to the animal.

Genus *Gasterophilus* (parasitic in horses).

The larva of *Gasterophilus* is reddish in colour, due to the presence of a pigment similar to the haemoglobin found in certain chironomid larvae. This larva was believed by Buchner (1922) to feed on blood extracted from the mucous membrane of the stomach, to which it remains attached. It has now been definitely demonstrated that it feeds entirely on the stomach contents of the horse, as a consequence of which the health of the animal is not affected, even in the presence of a heavy infestation. (Roy, 1937).

Gasterophilus haemorrhoidalis L. (horse-fly): is very annoying to horses at the time the eggs are laid. The eggs are deposited singly in the pores of the minute hairs of the lips; they hatch in 5 to 10 days. The larvae are ingested with food or water, and attach themselves to the walls of the stomach, where they remain for a long time but later migrate to the rectum where they reattach themselves. Before leaving the host they usually attach themselves close to the anus and protrude from it. They drop off after a certain length of time. The pupal stage lasts for 2 months. The length of life of the adult is from 1—7 days during which time they take no food but are very active, the female ovipositing throughout her existence.

G. nasalis L. (Throat bot-fly). Deposits its eggs on the hairs under the jaws and to some extent on the shoulders and other parts of the host. The larvae attach themselves to the wall of the pharynx, and later to the wall of the stomach and the duodenum.

G. intestinalis De G. (*equi* Clark): the common bot-fly. The eggs are deposited on all parts of the body but preferably on the forelegs. The larvae which develop upon the application of moisture and friction supplied by the rubbing and licking of the horse, are ingested and attach themselves to any part of the stomach.

Genus *Dermatobia*.

In common with others belonging to the family Oestridae the adult *Dermatobia* does not feed. The larva of *D. hominis*, L. is normally a subcutaneous parasite of cattle and of other domestic animals in Central and South America and the West Indies. Its presence in man is indicated by a painless tumour on the skin. An individual generally harbours one larva at a time and it is generally found in the arm or the leg though occasionally the face may be attacked.



Fig. 102
Larva of *Dermatobia*.
(After Blanchard)

The eggs are laid on leaves and branches of trees which become attached to the ventral surface of the abdomen of mosquito which mechanically transfers the first stage larva contained in the egg to the skin of animals. The mosquito most commonly involved is *Psorophora (Janthinosoma) lutzii* in Brazil.

Family Anthomyidae.

Members of the family Anthomyidae are characterised by the 4th longitudinal vein running outwards in such a way as to leave the 1st posterior cell widely open. The maggots are generally parasitic either on plants or on insects. One species, *Fannia canicularis*, L., also called the "lesser house-fly", which occurs in temperate climate, frequents dwelling houses though it is less attracted to food than is *Musca*.

The larvae breed in decaying and fermenting vegetable and animal matters and also in animal excrements. Larvae can be readily recognised from the spine-like processes on the thoracic segments.

F. scalaris Fab. The habits of this species are somewhat similar to those of *F. canicularis* L. but it prefers excrement as a nidus for the eggs and is very

commonly found breeding in human excrement. It is very common both in European countries and in North America. On account of its most common breeding habits, it may be called the "latrine fly". Although *F. canicularis* and *F. scalaris* bear general resemblance to each other, the two can easily be distinguished by the mid-tibiae which in *F. scalaris* bear a distinct tubercle which is absent in *F. canicularis*.

The larva closely resembles *F. canicularis*; the former is, however, provided with lateral processes which are feathered.

Several cases of intestinal and urinary myiasis have been reported in which the larvae of the above two species have been thought to be responsible. The validity of such findings has, however, been doubted.

BITING MUSCIDAE.

Genus *Stomoxys* (Stable fly).

It closely resembles house-flies and is found everywhere house-flies occur. It can, however, be quickly distinguished from the latter by its stiff proboscis. It is a medium-sized fly of a greyish colour. In most of the species the sexes can be differentiated by the approximation of the eyes; in males the eyes are closely placed whereas in females the eyes are widely divergent. The arista is plumose only on its dorsal surface. The proboscis greatly differs from the common *Musca*



Fig. 103
Larva of *Fannia scalaris*. (After Hewitt).

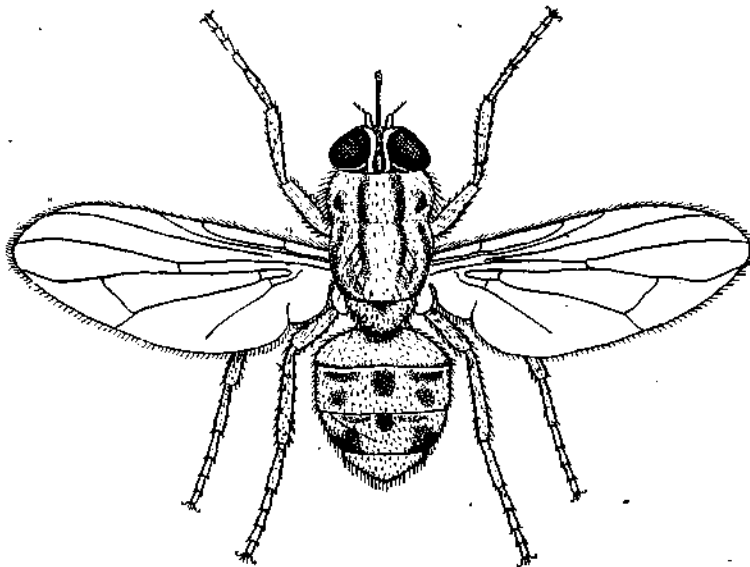


Fig. 104
Stomoxys calcitrans.

type. It encloses the sucking tube. Palps are slender and much smaller than the proboscis. The abdomen is differently marked in different species. They commonly attack domestic animals.

In the resting position the proboscis projects out like a bayonet. It is black, shiny and stiff. The labium is strongly chitinised and the labial gutter in which lie the hypopharynx and the labrum-epipharynx, is deep. These latter structures are united posteriorly. The labellae are greatly reduced and the pseudotracheae are totally absent. On the inner wall of the labella is located the cutting and scratching apparatus. These consist of about 5 teeth on each side and an interdental armature which is of a membranous character. The maxillary palps are less than half the size of proboscis.

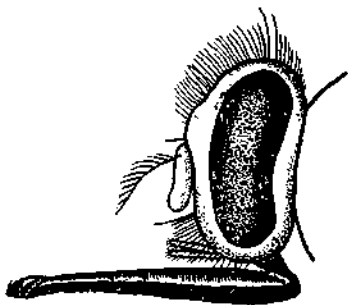


Fig. 105
Head of *Stomoxys calcitrans*.

Eggs are laid preferably in horse dung. The larva is smaller than that of the house-fly, the posterior spiracle presenting striking differences in form and in the position of the slits. Both males and females suck blood. In the tropics under the most favourable conditions the whole cycle may be completed in 12 days.

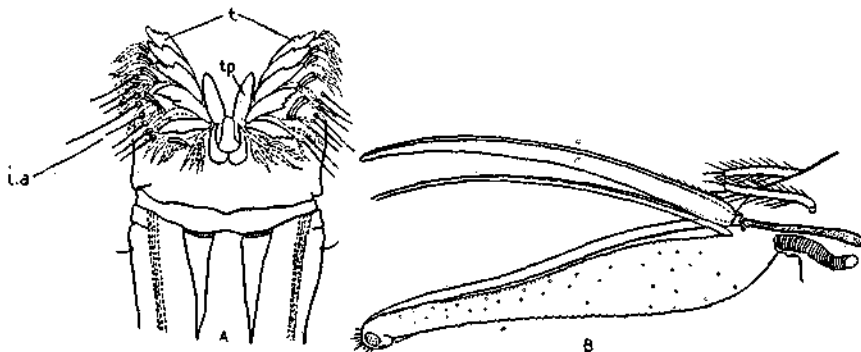


Fig. 106
Mouth parts of *Stomoxys*. (after Newstead).
A. Labella of *Stomoxys*.
l.a., interdental armature; t, teeth; t.p., tooth plate.
B. Labium of *Stomoxys* in the gutter of which labrum-epipharynx and hypopharynx are lodged.

S. calcitrans L.: is a very common species and is readily distinguished from others by the spotting on the abdominal terga. The presence of 6 round dark spots is a characteristic feature of this species. It is more closely connected with the horse than with the cattle. Although it is a true blood-sucker, yet it can be fed in captivity on syrup, or the liquid from decomposing vegetable matter or dung.

The frons in both sexes is wide, but the females are invariably the larger and of a lighter colour.

Adult *S. calcitrans* will often bite man while working in stables. It will even attack through thin clothing. The bite is painful but the pain soon passes off.

It has been definitely proved that *Trypanosoma evansi* may be carried in a mechanical manner by certain biting flies. There is no evidence to show that

any cyclic development of the organism takes place within the bodies of such insects.

Mitzmain (1912) was unable to transmit the disease through *S. calcitrans* except when the feeding was interrupted and restarted on some other animal. Duke (1912), on the other hand, found *Glossina palpalis* incapable of transmitting the disease. Nevertheless both Mitzmain (1912) and Kelser (1927) found *S. calcitrans* an inefficient transmitter of surra.

There is a distinct effect of fly attacks on milk production and the destruction of flies by spraying is always followed by an appreciable increase in the yield of milk.

For laboratory use *S. calcitrans* are bred in horse-dung. Langeron (1910) advises moistened bran which according to him is an excellent medium for breeding of larvae of *Stomoxys*. The bran should be previously sterilised. Darkness and moisture are essential for success. The flies may be induced to oviposit in tubes.

Insecticides are more useful for repelling these flies than for killing them.

Genus *Glossina*.

The tsetse flies or *Glossina* which are restricted to Africa, are the most important group of blood-sucking flies affecting man. They are the intermediate hosts of a fatal trypanosomal disease, the sleeping sickness in Africa, and also transmit *Trypanosoma brucei* which is the causative agent of Nagana in domestic animals.

They are readily distinguished from other flies by their characteristic antennae, wing venation and the proboscis. The method of reproduction too is unique. They are in reality larviparous, mature third stage larvae being passed which readily pupate after their discharge from the uterus of the parent fly.

The eyes are separated in both sexes.

Antenna. The antenna consists of 3 segments of which third is greatly enlarged. It has three olfactory pits on the 2nd and 3rd segments and numerous sensillae and spines. The lower anterior end of the third segment projects forwards. The arista bears on its dorsal surface a large number of long, curved and branching hairs.

Proboscis. The proboscis or the labium is rigid and is non-retractile. It is considerably enlarged into a bulbous expansion at its proximal end where it is attached to the oral sclerite. The pharynx runs along the ventral wall of the latter. At the distal end of the labium are the much reduced labellae. The proboscis is composed of the same parts as that of other blood-sucking Muscoidea. The sucking tube is composed of the dorsally placed labrum—epipharynx and the hypopharynx which lies ventrally. The common salivary duct passes through the entire length of the hypopharynx. Both epipharynx and hypopharynx are accommodated in the labial gutter.

The labellae are extreme distal parts of the proboscis and are fused ventrally. There are three rasps in each labellum and they bear rows of very sharp teeth. At the anterior end of each rasp there are four strongly sclerotised prestomal teeth.

The cutting and scratching apparatus consists mainly of the prestomal teeth and rasps.

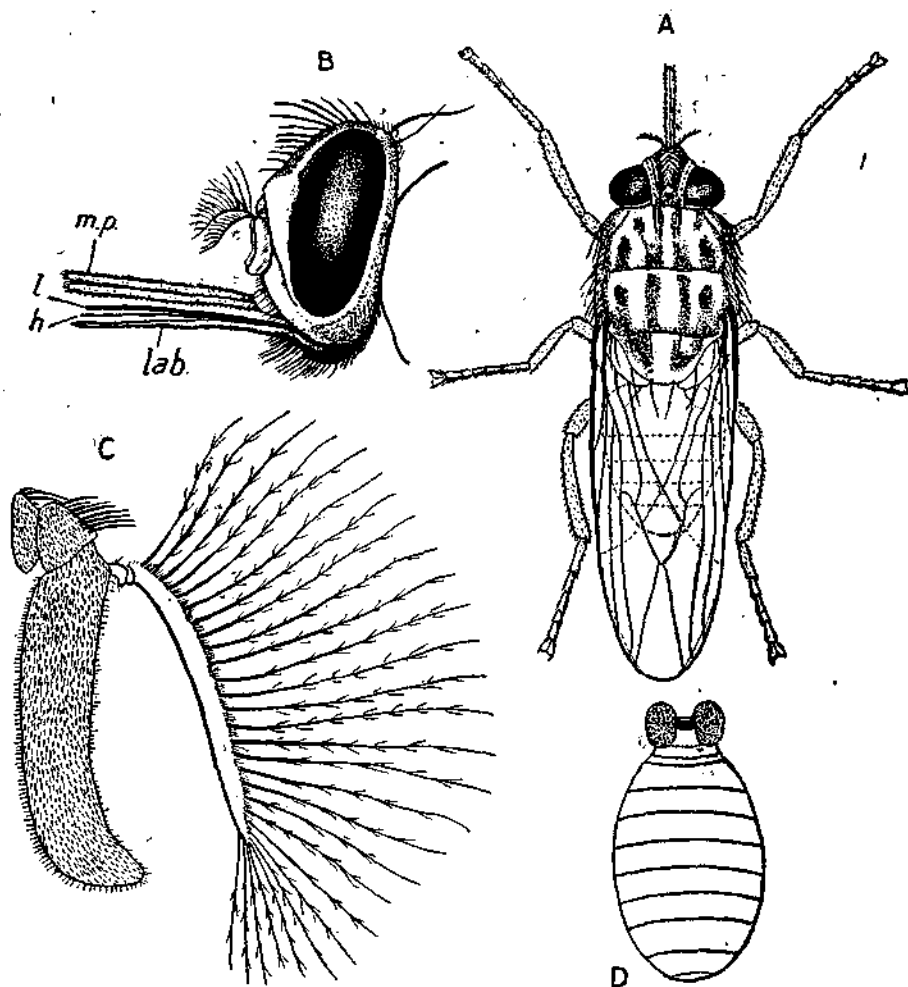


Fig. 107

- A. Resting attitude of *Glossina*. (After Austen).
- B. Head of *Glossina*.
mp., maxillary palp; lab, labium; l, labrum-epipharynx; h, hypopharynx.
- C. Antenna of *Glossina*. (After Austen).
- D. Pupa of *Glossina*. (After Castellani and Chalmers). (Placed upside down.)

Maxillary palps are long, even longer than the labium, well developed and covered over with spines and hairs. Normally the palps can not be distinguished from the proboscis.

Wing. When at rest the wings cross each other over the abdomen like the blades of scissors. The venation of the wing is in many respects different from *Musca* type. The veins are concentrated in the anterior part of the wing. The fourth vein ends at the costa at a point much anterior to the tip of the wing thus greatly narrowing the first posterior cell distally.

Alimentary canal. The alimentary canal is of the usual Muscoid type. The crop contains air-bubbles. The salivary glands are two very long and very much coiled tubes. The posterior end of the gland extends as far as the sixth abdominal segment. The ducts of each side pass ventrally behind the pharynx.

An anticoagulant function of the saliva was first suggested by Stuhlmann (1907) and its presence was demonstrated by Yorke and Macfie (1924) and subsequently by Lester and Lloyd (1928). The latter authors showed that the anticoagulin is in the nature of an antithrombin. They have also demonstrated that the fly deprived of its salivary glands can still draw blood, but sooner or later large clots form in the food canal of the proboscis and in the crop, so that the fly can no longer feed, and dies of starvation. The salivary glands of *Glossina* are free from digestive enzymes (Wigglesworth, 1929). The function of the salivary glands is therefore to prevent the coagulation of blood in the anterior part of the alimentary canal.

The proventriculus is as well developed and has the same function as in other Muscidae. The circular muscles act as a sphincter which prevents the regurgitation of the food once it has passed into the midgut. It also serves as a press in which the peritrophic membrane is produced and moulded (Wigglesworth, 1930). This gradually grows downwards. It thus forms a uniform membranous tube and extends as a chitinous sheath far into the midgut being interposed between the lumen of the gut and the epithelial lining. This is intact anteriorly but not so as it extends backwards.

The early development of the trypanosomes of *Glossina* takes place in the posterior part of the midgut between the peritrophic membrane and the stomach wall. As they migrate forward they are found outside in the lumen of the gut.

The gut of the tsetse fly contains certain elongated strips of greatly hypertrophied cells, in whose cytoplasm occur certain rod-shaped bacteria-like organisms called "bacteroids". These are carried from one generation to another, and are also found in many other insects. Their exact function is at present unknown.

Reproductive system. The hypopygium of the male is a large oval swelling which is anteriorly bisected by the anal slit.

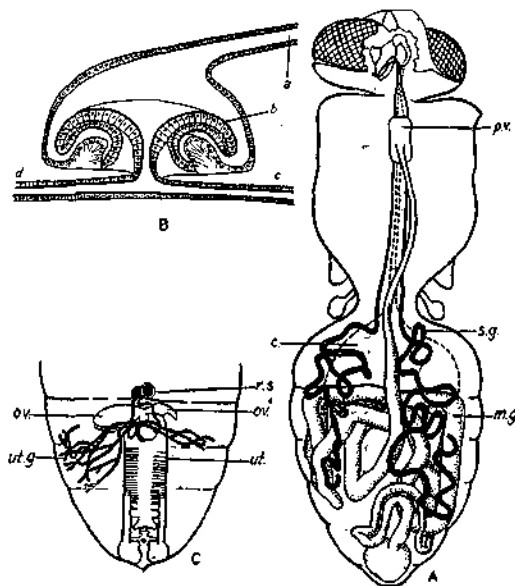


Fig. 108
A. Alimentary canal of *Glossina*. (After Minchin).
c., crop; mg., midgut; p.v., proventriculus; s.g., salivary gland.
B. Diagram of proventriculus of *Glossina* as seen in sagittal section. (After Wigglesworth).
a., midgut; b., invaginated portion of midgut; c., duct of crop; d., oesophagus.
C. Generative organs of female *Glossina*. (After Minchin).
ov., ovarioles; r.s., receptaculum seminis; ut., uterus; ut.g., uterine glands.

The reproductive system is different from the house-fly type. The ovaries are two in number and each ovary contains a single ovariole. The two ducts of the ovary open into a medially placed common oviduct. This is continuous with the uterus; the spermathecae are two in number and their ducts also open into the uterus. The accessory glands are two in number and each consists of branched tubular glands. They lie on the uterus, and the single duct of the two glands opens into the uterus. They secrete a milky white fluid which is thought to nourish the growing larva. The intrauterine life of the larva is about 10 days. The larva soon after its discharge from the uterus transforms itself into a pupa. The newly-born larva is covered with a slimy secretion which protects it against insects *e.g.*, ants. It also keeps the cuticle from being injured when the larva burrows into the soil.

The uterus contains only one larva at a time. The larva moults twice before it is discharged. The larva is yellowish-white in colour, segmented and bears two well-marked round protuberances posteriorly. These are separated by a depression in which the spiracles lie.

The puparium is dark brown and is of large size. The posterior protuberances in the larva persist in the pupa.

The minimum pupal period for *G. palpalis* and *G. tachinoides* bred in the laboratory during the rainy season has been recorded as 19 and the maximum, 46 days.

In the laboratory the female is not a prolific breeder as the number of progeny does not seem to exceed a dozen during her whole life time.

Glossina flies can be kept alive in the laboratory for several months. Males have a considerably shorter length of life than female flies.

They are active in the day time. Both species are blood suckers; some prefer big animals to bite, others prefer reptiles and birds. Sometimes they are found attacking snails and Lepidopteron larvae.

G. tachinoides West.—A small-sized fly. All the segments of the tarsus of the hind legs are entirely dark brown except at the base and tip of the first three dark segments of the tarsus which may be pale; a dark spot on either side of the second abdominal tergum. The third segment of the antenna is rounded at the end and has no long hairs along its anterior border.

It is widely distributed in West Africa extending from Senegal in the West to the shores of Lake Chad in the east; it is abundant in Northern Nigeria.

G. palpalis R-D.—All the tarsal segments of the hind legs are uninterruptedly dark; on the 2nd tergum there is a small pale triangle, the apex of which is continuous with a fine pale median line on the other terga.

This species is the most important among *Glossina* and is found in the northern, central and other parts of the Western Belgian Congo.

G. morsitans West.—The last two or three tarsal segments of hind legs are dark; last two segments of tarsus of front and middle legs with sharply defined dark brown or black tips, third antennal segment without any distinct fringe of hair on front margin.

It is the most widely distributed species of *Glossina* and next to *palpalis* it is the most important species.

LIFE HISTORY AND HABITS.

Among a large number of species of *Glossina*, only three are of considerable interest to us as they are concerned with the transmission of the parasite of sleeping-sickness to man. These are *G. palpalis* R-D. which is the vector of *T. gambiense* and *G. morsitans* West and *G. tachinoides* West, vectors of *T. rhodesiense*. Their distribution in Africa also varies. It has thus been possible to map out definite zones or belts in which a particular species is prevalent. The most important factors influencing the distribution and prevalence of tsetse-flies are the type of vegetation, the relation to game and the meteorological conditions.

G. palpalis is essentially a riverine species and is found along the banks of rivers where vegetation is dense. *G. morsitans* is seldom found near rivers and is more abundant in the savannah forest as well as in the open country where small water-holes exist, probably because game comes to water there; this species undoubtedly migrates by following herds of game for long distances. *G. tachinoides* is an upcountry form allied to *G. palpalis* and is found in similar localities on the higher reaches of the rivers.

The food of *Glossina* appears to consist wholly of blood of both mammalian and non-mammalian animals. *G. morsitans* has a tendency to feed on mammalian blood, *G. palpalis* ingests both mammalian and non-mammalian blood, while *G. tachinoides* shows a preference for feeding on reptiles and bats.

G. tachinoides and *G. palpalis* have the same breeding habits, the most common situation being in the decaying humus beneath overhanging trees, where the sun seldom or never penetrates and the ground is never dry. Their breeding places are always situated in close proximity to water.

For the breeding of *G. morsitans* only a small amount of shade provided by undergrowth is necessary. The condition of the soil is also an important factor in determining the breeding places. Pupae are deposited also in cavities of trees. The favourite situations are near water-holes.

G. tachinoides and *G. morsitans* are found in or near bushes, while *G. palpalis* may frequently be found in close proximity to villages.

The density of *G. morsitans* is greatest at the end of the heavy rain. During the dry season there is a distinct drop in the density of the adult fly. Large game movements cause a definite reduction of the female population. The fly seeks its host entirely by sight and if the antennae are rendered functionless, the fly is less ready to feed. These flies are greatly attracted by moving games and other moving objects, such as motor vehicles, cyclists, pedestrians etc. and follow them for long distances, and having fed, they make for the nearest shady woods and digest their meal (Nash, 1930). The flies disperse when the object they are following comes to rest. An abundance of these flies is correlated with the actual or recent presence of game in any given area. As a rule the high development of the sense organs connected with the antennae renders tsetse-flies able to detect the food from a long distance. A meal is required every five or six days, hence the presence of a number of game animals is not essential.

The females of *Glossina* usually take a large blood meal about 3 days before larviposition and do not feed again until the larvae have been deposited.

The flies are active in the day time and seldom attack their prey at night though reports to the contrary have been published.

A marked disparity exists in the proportion of the sexes of *Glossina*.

Flight experiments indicate that the greatest distance covered by a single female *G. tachinoides* is 4 miles and it is undoubtedly found farther from its breeding places than *G. palpalis*.

Developmental phases of Trypanosome.

Trypanosomes may even die off in the intestine. When established in the mid-intestine, they multiply in the posterior part. After the 10th day these forms become all sizes and shapes. They now increase in enormous numbers from the 10th to the 15th day after the infective blood feed. They migrate forwards in large numbers to the proventriculus. They are found in this situation from the 20th day onwards. They now make a forward movement into the oesophagus, pharynx and into the buccal cavity. From here they enter the salivary duct and pass backwards along the common duct into the two salivary glands where the polymorphic forms are seen. Thus infection may take place directly from the buccal cavity or from the salivary secretion at the time of biting.

Mechanical transmission of *Trypanosome* of sleeping-sickness can not be totally ruled out though under ordinary conditions the chances are small. It is, however, thought that when the disease occurs in an epidemic form, the flies transmit the parasites mechanically. This can take place only if the transference of the flies from the infected to the healthy animal is instantaneous, *i.e.*, by interrupted feeding.

In regard to the sites where the multiplication of various trypanosomes occurs, different species show great variations in this respect. Thus *T. congolense* (a parasite of cattle in Africa), *T. cruzi*, and *T. lewisi* multiply in the stomach only. In addition to the stomach, multiplication also takes place in the salivary glands in the case of *T. gambiensi*, *T. rhodesiensi* and *T. brucei*, while *T. vivax* multiplies in the labrum and hypopharynx.

The method of transmission also varies widely in different species. Though the usual method is by the bite of the insect, faecal contamination or crushing and ingesting the crushed insect are the methods of infection in the case of *T. lewisi* and *T. cruzi*. *T. equiperdum*, on the other hand, is transferred from one horse to another by sexual intercourse without the intervention of any outside agency.

The different transmitting agents of trypanosomes are:—(a) *Glossina*, (b) Tabanids, (c) Fleas, (d) Rhynchota or bugs and (e) Leeches.

Prevention.

The destruction of *Glossina* by artificial means is by no means easy.

G. palpalis is essentially a riverine species on the cattle route and can be restricted by clearing the vegetation. *G. morsitans* is restricted to the tall vegetation and fringing shade of rivers and small streams. In consequence of clearing the land and driving off the big game, the zone may become to a great extent cleared of flies. Such thinning of the forest is likely to be of value if it is intelligently

carried out in appropriate places frequented by man. No permanent fly breeding zones should however be attacked.

Marked improvement in many places has resulted from the systematic collection of pupae from breeding places.

Shircore (1916) suggested a method of trapping *Glossina morsitans*, based on the known habits of this fly. The traps consist of revolving canvas screens smeared with adhesives which are placed on routes known to be frequented by flies. A very large variety of traps have since been devised.

In fly infested territories, journeys should be undertaken only at night. No habitation within 500 yards of fly-breeding zones should be allowed to exist and the intervening area must be cleared of jungle and undergrowth.

Although several natural enemies belonging to species of Hymenoptera have been recorded parasitising pupae of *Glossina* the chances of their being utilised for the purpose of controlling these flies are remote.

Genus *Lyperosia*.

Lyperosia flies are cattle pests and are distributed all over the globe. They possess biting and piercing mouth organs as in *Stomoxys*. In size they are much smaller than the latter and are generally yellowish grey in colour. The proboscis is very slender, and projects downwards. The maxillary palps are very long. When at rest the position of the wings is scissor-like, and they are kept folded over the abdomen. The thorax has some inconspicuous longitudinal markings. The 4th longitudinal vein has a more gentle upward curve than in *Stomoxys*. Eggs are reddish-brown in colour and deposited in fresh animal dung, generally of the animal on which the adults are parasites.

L. irritans L. Occurs essentially in Europe and America. It is a comparatively large species; palps and legs in *L. irritans* are dark-brown to black. Unlike *Stomoxys calcitrans* it is never found in large numbers in stables. It feeds only on the blood of horned cattle on whose bodies it remains constantly even while resting, hence they are called horn flies. On one animal as many as 1,000 individuals may be seen. The shortest period between egg to adult is 17 days. Eggs are deposited in cattle dung.

L. exigua Meij. This fly is widely prevalent in Asia. It is a large species, in fact, one of the largest in this genus. It is slate grey in colour; thorax with 2 narrow well separated stripes; abdomen with a dark longitudinal median band on the first and second segments. The legs are yellow. It is widely distributed in India. The fly is commonly seen sitting on the shoulders of cattle with its head directed downwards.

The eggs are laid singly to the number of 10 to 20 in dung; the larvae emerge in 18 to 20 hours. Larval life is 3 to 4 days; pupal period 3 to 5 days.

L. minuta Bezzi.

A small dark grey species which has five or six hairs on the arista. The thorax is brown with two brown lateral stripes, and the abdomen is dark grey without any distinct markings. The legs are pale. The first posterior cell is very narrow distally. This fly is present throughout India, and resembles *L. exigua* in the breeding habits.

BITING FLIES AND DISEASES OF ANIMALS

Rinderpest.

This disease has always been present in Asia. Although it once existed all over Europe and caused enormous loss of cattle but it has now been completely suppressed. The exact mechanism of its spread is not known. There is, however, strong evidence to indicate that this virus disease of cattle is spread by flies. Bhatia (1935) has succeeded in reproducing the disease by interrupted feeding with *Lyperosia* sp., and Kapur (1941) with *Tabanus orientis* Wlk., though mosquitoes, ticks (*Boophilus annulatus*) and *Stomoxys calcitrans* were found incapable of transmitting the disease. (Sen, 1926 ; Sen and Salam, 1937).

Haemorrhagic septicaemia.

This is a plague-like disease in animals and biting insects especially flies, have been suspected to carry it in nature (Sen, 1925).

Fowl-pox.

Kligler and his co-workers (1929) have pointed out that the disease can be experimentally transferred by the bites of various species of mosquitoes and that the virus remains active for a considerable period in the body of the insect after it has once become infected. Biting flies may in the same way be responsible for the carriage of the virus from diseased to healthy animals.

Anthrax.

Anthrax is another disease which may be carried by both biting and non-biting flies. In such cases the fly acts merely as a mechanical agent. It carries the bacilli on its body and on the labellae which are contaminated by its vomit.

MYIASIS

Myiasis is the condition where any part of the body of man or animal is invaded by eggs or larvae of Dipterous flies. The larvae may be found either in any of the natural orifices, on wounds on the skin, in the alimentary canal or in the tissues of the body. Patton has placed myiasis-producing flies into three groups: (a) obligatory (b) facultative and (c) accidental.

Obligatory.

Here the larvae will only develop on living tissues of animals, also of man. The following is a list of obligatory myiasis producing flies.

Oestrus ovis, L.: widely distributed ; affect nasal sinuses of sheep and on rare occasions the conjunctiva of shepherds.

Hypoderma bovis, DeG: the common warble-fly of cattle in Europe.

H. lineatum, Vill.: the warble-fly of cattle in America.

H. crossi, Patt: the true warble-fly of goats and sometimes of cattle in the Punjab in India.

Dermatobia hominis, L.: normally a subcutaneous parasite of cattle in Central and South America. Sometimes attacks man in the same manner.

Auchmeromyia luteola, F.: restricted to Africa and its larvae are considered to be specific human parasites as they live on the blood of man.

Cordylobia anthropophaga, Grün.: the larvae form subcutaneous tumours in domestic animals and also in man in Africa.

Cochliomyia hominivorax, Coq.: widely distributed in America and West Indies: it is the typical screw-worm fly of the New World. It attacks generally one of the natural orifices of man. It causes external myiasis in animals.

Chrysomya bezziana Vill.: the screw-worm fly of Africa and India; in man its larvae are found in one of the natural orifices of the body. On animals they are found especially on the scrotum after castration.

<i>Gasterophilus nasalis</i> L.	} The larvae of these three species of <i>Gasterophilus</i> are found in the alimentary canal of horse
<i>G. haemorrhoidalis</i> , L.	
<i>G. intestinalis</i> , DeG.	

Facultative.

Sarcophaga species: on external wounds of man and animals all over the globe.

Wohlfahrtia magnifica, Schin.: external myiasis in animals in Southern Russia.

<i>Lucilia sericata</i> , Meij.	} These are the blow-flies of sheep in Europe and Australia.
<i>L. caesar</i> , L.	

<i>Chrysomya rufifacies</i> , Macq.	} They also attack sheep in Australia.
<i>Pollenia stygia</i> , F.	

Phormia regina Mg.: known as the black fly which is a well-known pest of sheep in many parts of Canada and the United States.

Chrysomya macellaria Fab.: cause external myiasis in domestic animals in Mexico.

Accidental.

Diptera larvae may occasionally be noticed in human stools. They are generally ingested with the food and are passed in a dead condition. Among them larvae of *Eristalis* may at times cause unpleasant intestinal symptoms especially in children and lunatics. *Apiochaeta scalaris* Mg. is believed capable of passing its life cycle in the human intestine, living larvae being passed from time to time.

It is extremely doubtful if Muscoid larvae can survive in the intestinal canal of man and pass unchanged in the faeces as the experimental works of Causey (1938), and Strickland and Roy (1940) clearly indicate. Regarding living larvae of *Apiochaeta scalaris* passed in the human stool, it may be pointed out that such cases, that had come to our notice, occurred during the most favourable time, i.e., summer, when the developmental process was very rapid. The larvae were all of one size, were immature and were seldom intimately mixed with the stool.

The following is a list of myiasis-producing flies in man in India recorded by Strickland and Roy (1941).

Eye: *Anthomyidae*, Sp.

Nose: *Chrysomya bezziana*, Vill.

Sarcophaga, Sp.

Ear and dental sockets. *C. bezziana*, Vill.

Mastoid region: *Sarcophaga dux*, Thos.

Skin: *S. ruficornis*, Fb.

Intestinal: *S. ceylonensis*, Park.

S. ruficornis, Fb.

S. dux, Thos.

S. craggi, Park.

Drosophila, Sp.

Apiochaeta scalaris, Mg.

Treatment of Myiasis.

Human myiasis. Protection of the wound with gauze will prevent flies from ovipositing on the wound. Where there is any foul-smelling discharge from the nostril, mouth, etc., adequate treatment should be carried out. Where infestation has actually taken place, the parts should be painted with a thick layer of castor oil with the idea of suffocating the larvae and forcing them to appear on the surface. Where possible, the affected parts may be kept immersed in water or any antiseptic fluid for 15 minutes to half-an-hour. The larvae can then be picked up with forceps and destroyed. Sometimes it is not possible to adopt any measure whatsoever. Beyond the fact that the presence of fly larvae on one's own person is extremely repugnant to him, no ill effects will result from their presence alone. It may be pointed out that the maggots will appear on the surface and leave the wound as soon as they are mature with a view to pupation which always takes place outside the body. Ordinary antiseptics have no effects on larvae.

Animal myiasis. Animal myiasis should be treated with a powder composed of D.D.T.—5 parts and Boracic acid—95 parts. Particular attention should be paid to the cleanliness of the wound in order to prevent reinfestation. Pyrethrum powder can be used in place of D.D.T. If any of these two insecticides is not available, phenyle may be used.

Maggot Therapy.

Although it was known that maggot infested wounds heal quickly, it was not till Baer (1931) demonstrated the value of blow-fly larvae in the treatment of osteomyelitis and of other infected wounds, that maggot therapy was established as a useful surgical procedure. When placed in infected wounds, maggots decrease the bacterial population and remove necrotic tissue. Wounds treated with maggots become sufficiently alkaline to turn red litmus blue (Baer, 1931). The liquefaction of the necrotic tissue is thought to be effected by a trypsin-like protease found in both the digestive tract and excreta of the larva, and by collagenase in the excreta (Hobson, 1931). Larvae of *Lucilia cuprina* and *Chrysomya rufifacies* are capable of liquifying and digesting protein without the intervention of bacteria (Mackerras and Freney, 1933). Aqueous solution of excreta of larvae of *L. sericata* and possibly of other species has been shown by Simmons (1935) to contain potent bactericidal properties.

Thus certain species whose maggots attack only dead tissues can be advantageously employed in the treatment of surgical conditions such as cellulitis, osteomyelitis, etc. For this purpose the maggots should be bred under very clean conditions and released on the wound as soon after their emergence from the

egg as possible. In India larvae of *Chrysomya megacephala* have been used for the treatment of cellulitis of the lower extremities of two patients whose general conditions did not permit any extensive surgical measures to be carried out. Two narrow openings were made for irrigation of the wound with antiseptic fluids. A large number of first stage larvae at a time were released and this was repeated once after a week. Under the combined treatment with maggots and irrigation, the wound healed up very quickly. Muscoid larvae are extremely resistant to antiseptics in the strength they are commonly used, and therefore the larvae make no attempt to escape. Pupation always takes place on the ground and never in the wound.

Other flies which have been employed in maggot therapy elsewhere are *Lucilia sericata*, *L. caesar* and *Phormia regina*. They will feed on dead tissue and breed in animal matter.

The procedure of breeding flies for therapeutic purposes is as follows. The flies are induced to oviposit on meat and the eggs are allowed to harden in cool surroundings for twenty-four hours. They are placed on some glass wool in a funnel and washed for about five to ten minutes with either mercuric chloride solution (Bogdanow, 1906), or a mixture of 5 per cent solution of formaldehyde with 1 per cent solution of sodium hydroxide (Robinson, 1934). The eggs are next washed with water and dropped in a flask containing sterile ox liver. If the flask is kept in a warm place, this will hasten emergence of the larvae. The larvae are picked up with a sterile camel hair brush and washed in water before they are transferred to the wound.

Rearing larvae from cases of myiasis.

In spite of the presence of a large number of larvae, it may take some time to extract a few as the larvae are very active and may burrow deeply. The larvae may be recovered by exposing the affected part to the vapour of chloroform or better by covering it with melted paraffin. The latter method is adopted with the purpose of partially asphyxiating the larvae, when they will readily appear on the surface. One by one they will have to be picked up with forceps. A few may be killed in hot water and preserved in 80 per cent spirit and the rest reared to the adult stage. For this purpose meat is used as the pabulum. It must be flamed or just placed in boiling water to ensure freedom from other larvae. The meat is now placed on a thick layer of sand in a deep vessel or a tin and the larvae are dropped on the meat. The opening of the vessel should be covered with two or more layers of muslin to prevent other flies from depositing their eggs or larvae on the meat. Pupation takes place in the sand. The pupae may be placed in test-tubes plugged with cotton wool till the flies hatch out.

On no account should bred-out flies be killed within 24 hours after emergence.

In the case of the specific myiasis-producing Diptera, it is useless trying to rear the immature larvae on meat. Only third stage larvae should be allowed to pupate in sand.

Identification of larvae from cases of myiasis.

Specific identification from examination of larvae alone is by no means easy. An attempt may be made to identify the genus from (1) the posterior spiracles,

(2) intersegmental spines, (3) anterior spiracles, and (4) the pharyngeal ridges. The character of the cephalopharyngeal skeleton may be of considerable help in the identification.

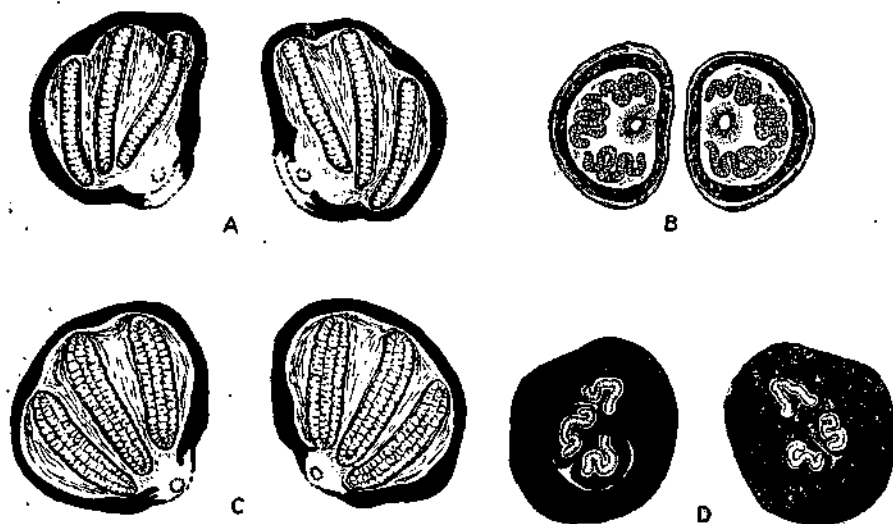


Fig. 109 .

- | | | |
|----|------------------------|--------------------------------|
| A. | Posterior spiracles of | <i>Sarcophaga ruficornis</i> . |
| B. | " " | <i>Musca vicina</i> . |
| C. | " " | <i>Chrysomya megacephala</i> . |
| D. | " " | <i>Stomoxys calcitrans</i> . |

PUPIPARA (Tick Flies).

Genus *Hippobosca*.

These are all degenerate Diptera which have adapted themselves to more or less continuous existence on the bodies of birds or mammals. They are sometimes called Pupipara on account of their peculiar mode of reproduction. It is thought that they give birth to third stage or mature larvae which soon after their extrusion are changed into pupae. But it must be remembered this method of reproduction is not confined to this group alone.

These flies all possess a shining hard cuticle which is also ornamented in many species. The head is impacted in the thorax. The antennae are greatly reduced. The segmentation of the abdomen may or may not be present. Wings may be present or absent. The legs are stout.

All the known species are included in three families, *Hippoboscidae* found on mammals and birds, *Nycteribiidae* and *Streblidae* on bats.

The Family Hippoboscidae includes the three important species which are of some interest to us.

(1) *Melophagus ovinus*, the sheep tick-fly, are especially injurious to young lambs, to which they migrate in large numbers when the older sheep in a flock are sheared.

(2) *Pseudolynchia maura* are ecto-parasites of the pigeon, and have been shown by Sergeant brothers (1908) to be the vector of *Haemoproteus columbae*.

This fly is viviparous and is cosmopolitan in its distribution. The pupae are deposited in birds' nests and are affected by kerosene-pyrethrum mixture. The pupal life is nearly a month. In this species the wings are of a peculiarly pointed form though they are rounded at the tip.

(3) *Hippobosca maculata* is a common species found on horses, dogs etc. The larva is white in colour but it soon becomes shining black which is an indication that the larva has pupated. It has been stated that the pupae occur in cowdung, and horsedung. It is perhaps more correct to say that the flies deposit their larvae on the ground, and sometimes perhaps in holes of trees (Stekhoven, 1926). This species is believed to be capable of transmitting trypanosomiasis mechanically like other biting flies.

In *Melophagus* both wings and halteres are absent; in *Lynchia* wings are of a peculiarly pointed form, the tips however are rounded. In *Hippobosca maculata* wings are always present.

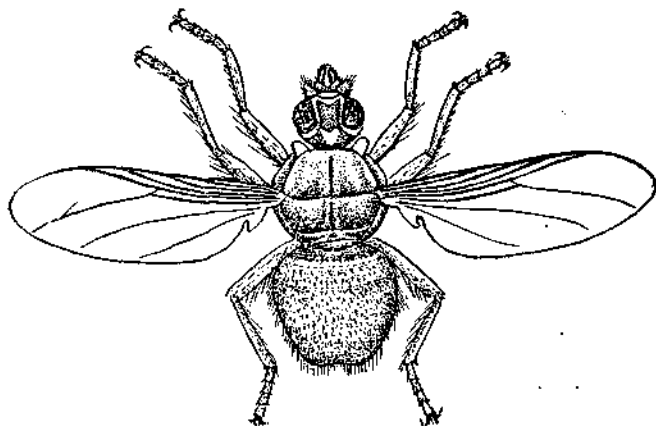


Fig. 110
Lynchia maura, female.

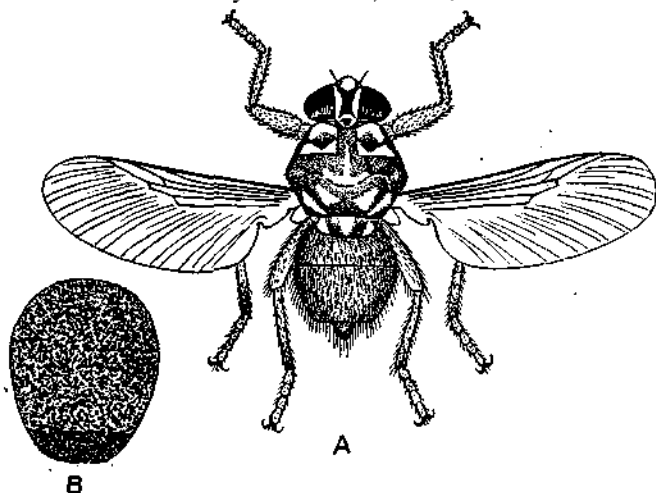


Fig. 111
Hippobosca maculata. A, adult; B, pupa.

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SIPHONAPTERA

	Order Siphonaptera		
Families	Pulicidæ (Rat fleas)	Sarcopsyllidæ (Chigger fleas)	Ceratopsyllidæ (Bat fleas)
Genera	<i>Pulex</i> <i>Xenopsylla</i> <i>Hoplopsyllus</i> <i>Nosopsyllus</i> (<i>Ceratophyllus</i>) <i>Ctenocephalides</i> (<i>Ctenocephalus</i>) <i>Pygiopsylla</i> <i>Leptopsylla</i> (<i>Ctenopsylla</i>) <i>Hystriopsylla</i> <i>Ctenophthalmus</i>	<i>Echidnophaga</i> <i>Tunga</i> (<i>Dermatophilus</i>)	

Fleas belong to the order Siphonaptera which comprises a large number of insects which are distributed all over the world. Some of them are of particular concern to us as they act as intermediate hosts of the parasites of bubonic plague. Fleas are found on many warm-blooded animals, and they are parasites only during the time they feed on the host. Although they are regarded as free parasites, there is one family, Sarcopsyllidæ, in which the females after impregnation attach themselves permanently to their hosts.

The chief characters of this order are: (i) the body is laterally compressed so as to enable the insects to move freely in the hair of the host; (ii) the cuticle is strongly chitinised; (iii) the adult is wingless, hence it is incapable of flying; (iv) both sexes lead parasitic lives on warm-blooded animals including birds; (v) the mouth parts are adapted for piercing and sucking blood which constitutes the only food of both sexes; and (vi) metamorphosis is incomplete, the larva being caterpillar-like and the pupa enclosed in a cocoon.

External structure.

The body is heavily chitinised and the thorax is attached to the head without the intervention of a neck.

Head: The head is roughly conical in shape and is divided by an antennal groove into an anterior and a posterior part, known as the front and the occiput respectively. The lateral lower portion of the front beneath the eye is the genu or the cheek which is wide and in some species there is a row of strongly chitinised teeth or comb arranged in a symmetrical manner along its free edge. The eyes may be present, absent or rudimentary. The antenna at rest lies in an antennal groove which is situated above and behind the eyes. The antenna consists of three parts, the terminal one being club-shaped and may be completely or incompletely divided into a number of smaller segments. There are some bristles on the head which are of taxonomic importance.

The mouth parts are conspicuous structures and project from the head downwards. They consist of (1) a pair of maxillae, (2) a pair of maxillary palps,

(3) labium, (4) a pair of labial palps, (5) a pair of mandibles and (6) the labrum. The maxillae are two triangular plates with an acute angle at the free end. They are attached to the lower edge of the head. The maxillary palp projects like antenna

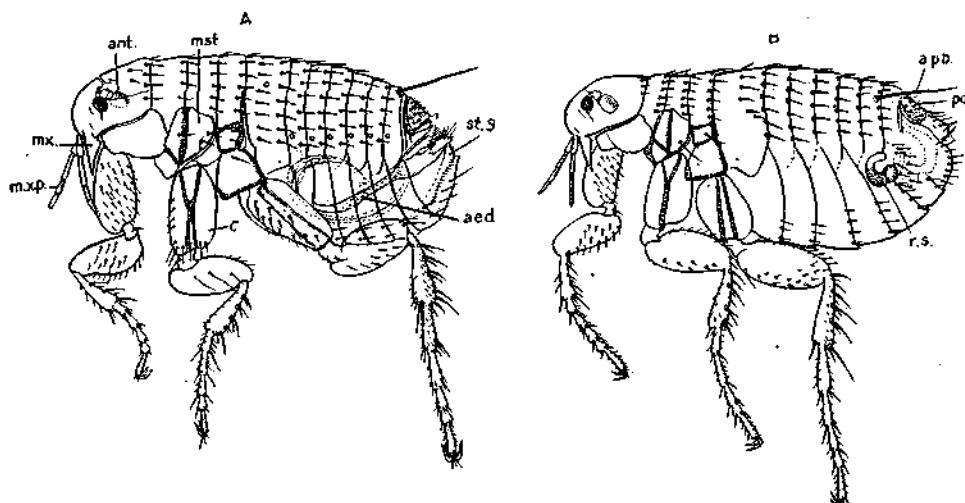


Fig. 112

A. *Xenopsylla cheopis*, male. B. *Xenopsylla cheopis*, female.
aed, aedeagus; ant, antenna; apb, antipygidial bristle; mst, mesosternite;
mx, maxilla; mxp, maxillary palp; pd, pygidium; rs, receptaculum
seminis; st. 9, 9th sternite.

from the lower front of the head and is a four-segmented structure. The labium is small and insignificant, and to it is attached the labial palp which is also segmented. The mandibles are long, needle-like and finely serrated. The labrum-epipharynx is also needle-like and barbed, and pointed at the end.

The actual cutting organs are the finely serrated mandibles and possibly the apex of the triangular maxillae. The sucking tube is formed by the apposition of the mandibles and the labrum-epipharynx.

Thorax: The three thoracic segments are quite distinct. In some species the pronotum has a row of chitinised teeth or comb along its posterior border.

The legs are very powerful, the coxa being greatly enlarged. The trochanter is very small. The tarsi consist of 5 segments, the last segment having a pair of stout claws.

Abdomen: The abdomen is really composed of 10 segments but only 9 are clearly discernible, the last 3 being modified for sexual purposes. The 9th tergum bears a pitted and setose sensory plate known as the pygidium whose function is at present unknown. Near the posterior margin of the 7th tergum there is a pair of prominent stout bristles, one on either side of the middle line, which project over the pygidium; these bristles are known as antipygidial bristles.

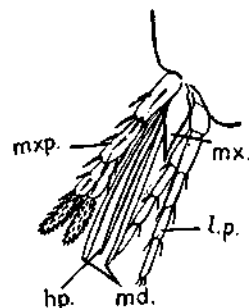


Fig. 113
Mouth parts of
Xenopsylla astia,
hp, hypopharynx;
lp, labial palp;
md, mandible;
mx, maxilla;
mxp, maxillary palp.

The male flea is characterised by the presence of the aedoeagus or penis which is a complex structure and lies coiled up inside the abdomen. There are two pairs of claspers. The 9th sternum lies for the most part inside the abdomen and only a small part projects out from it. In mounted specimens the 9th sternum appears to be covered by the sternum of the 8th segment.

In the female the receptaculum seminis is a striking object and is situated about the middle of the distal end of the abdomen. On pressure it is liable to be displaced. It is arbitrarily divided by a deep constriction into a highly chitinated round portion called the "head", and the rest being called the "tail". Its shape is of considerable significance in the identification of the flea.

There are three pairs of thoracic and seven pairs of abdominal spiracles. The first abdominal spiracle is wanting.

Internal anatomy.

The pharynx is provided with a powerful pump for sucking blood from the wound. The proventriculus has on its inner surface a number of chitinous rods

which project into the cavity, and in the ordinary circumstances they act as a valve which prevents the regurgitation of ingested blood.

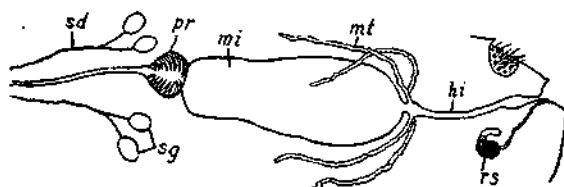


Fig. 114

Alimentary canal of *Xastia*.

hi, hindgut;
mi, midgut;
mt, malpighian tubules;
pr, proventriculus;
rs, receptaculum seminis;
sd, salivary duct;
sg, salivary glands.

The midgut is a short saccular tube which when fully distended occupies almost the whole abdomen. The hind intestine is extremely narrow and short. At the junction of the stomach and the intestine are four Malpighian tubules. The rectum

contains six papillae. The anus opens externally below the 9th tergum.

There are two pairs of small round salivary glands and the common salivary duct runs in the head and enters the salivary pump.

Reproductive system. In the male it consists of (1) a pair of testis with the corresponding vas deferens which opens into a single tube; (2) the seminal vesicle from which (3) the ejaculatory duct leaves to enter the penis or aedoeagus; (4) there are in addition two pairs of accessory glands which open into the vas deferens of each side.

The female reproductive organs consist of (a) ovaries, each consisting of a number of independent tubes containing egg follicles and converging to form a single tube; (b) the common oviduct; (c) the vagina; and (d) the receptaculum seminis or spermatheca.

Life history and Bionomics.

The flea has a complete metamorphosis, the successive stages in its life being egg, larva, pupa and adult. The egg is oval, transparent and on account of its

softness it is liable to be damaged on handling. It has the tendency to adhere slightly to the material on which it is laid. Its length is about 0.6 mm. and though small, it is visible to the naked eye. The eggs are deposited in places where the host rests. They are never laid on the body of the host. For egg-laying the female usually selects dark and dry places such as rubbish, debris and dust in domestic houses, under carpets, in granaries etc.. Rat-fleas often lay eggs in rat holes, bird-fleas in birds' nests, and dog and cat-fleas where these animals lie at night. The female generally lays a few eggs at a time and provided it has access to frequent blood meals, it will continue laying throughout its life time. The number of eggs laid by a single female during its life time is not known but it is thought that a single individual can lay at least 200. Strickland (1914) working with *Nosopsyllus fasciatus* found that the eggs are laid even at a low temperature as low as 50°F.

During the summer in the tropics the eggs do not take more than 4-5 days to hatch. In common with other insects a low temperature proportionately prolongs the incubation time of the egg.

In unfavourable conditions the eggs may even take 9 days. Ova of *X. cheopis* do not hatch at temperatures below 55°F, and the optimum is attained at 80°F and saturation deficiency 0.23 inches.

The incubation period of *Xenopsylla astia* eggs is distinctly less than that of *X. cheopis* while *X. brasiliensis* on the whole hatch earlier than either.

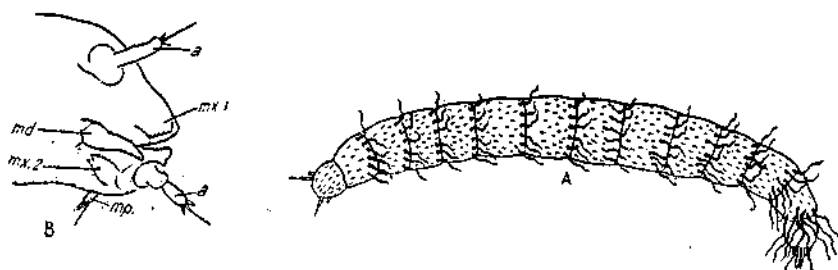


Fig. 115

A. Larva of *X. astia*.

B. Mouth parts of larva of *X. astia*.

a. antenna; md, first maxilla; mx. 2, second maxilla; mx. 1, mandible; mp, maxillary palp.

The larva is a footless hairy caterpillar-like maggot, extremely active, and is of a whitish colour. The body consists of a distinct head, three thoracic and ten abdominal segments. The head carries a pair of antennae, a pair of toothed mandibles and two pairs of maxillae with jointed palps. In *X. cheopis* there are two rows of hairs, an anterior row of small hairs and a posterior row of larger ones on each side of the thoracic and abdominal segments. The last abdominal segment is very small and possesses a pair of chitinous processes which are of use to the larva at the time of locomotion. The anus is situated on a raised area of chitin which is sunk into the tenth segment.

The larva, though it is devoid of eyes, is yet very sensitive to light, especially in its younger stages. It is rapidly killed when exposed to direct sunlight.

Bacot and Ridewood (1914) found that the chief food supply of larvae of most species is the excreta of the parents. It is believed that the larvae of many species of fleas require blood in their food and without it, their growth will be retarded. There is always the appearance of a dark reddish line in the alimentary canal of very young larvae suggesting the ingestion of blood by them. In nature the blood is supplied by the adult fleas, which swallow more blood than they can digest, so that some of it is discharged along with faeces. Blood, although it may constitute the most important part of the nutritional requirements of the flea larvae, is by itself not sufficient for their normal and successful development. The flea larvae require an additional food which in nature is supplied by the organic refuse present in the bed of the host of adult fleas (Sharif, 1937).

As it is always possible to breed *Xenopsylla astia* and *X. cheopis* exclusively on dried animal faeces without any blood, it seems reasonable to suppose that in nature organic refuse matters are sufficient to provide the larva with the necessary food requirements.

The flea larva moults twice and has three larval stages. The first larval instar can be distinguished from the other stages by the presence of a hatching spine on the head. The size is useful in distinguishing the other two stages. The third stage larva defecates whereby its intestine becomes clear of all food taken during the larval period. After defecation, the larva becomes active and restless. Later it starts spinning a cocoon and becomes bent in the middle so that the head and the tail ends come to lie close to each other. It soon becomes inactive and it is said to be in the resting stage. If the cocoon is opened at this stage the larva will not move, but will pupate without the cocoon under suitable conditions of temperature and humidity. This resting stage has been called prepupa by Mellanby (1933).

The flea larva is killed not only by high temperature but by low humidities at all temperatures on account of lack of protective mechanism against excessive loss of water. At high temperature in the presence of suitable humidity the larva grows more quickly.

The larva of *Nosopsyllus fasciatus* thrives best at a constant temperature of 83°C and 80 per cent relative humidity.

The normal length of life of a larva is approximately a fortnight.

The cocoon is made of silken material to which particles of sand, grit etc.

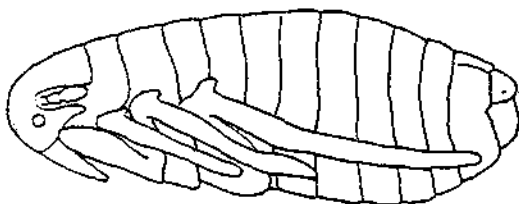


Fig. 116.
Pupa of *Xenopsylla astia*.

adhere. The eyes and antennae are clearly visible. The legs remain folded against the body; the segmentation of the body is also clear. Sharif (1935) has recorded the presence of wing buds on the mesothorax in the pupa of *Nosopsyllus fasciatus* but found them absent in *Xenopsylla cheopis*. The pupal

stage usually lasts from 7 to 15 days. It may, however, be prolonged for a considerable length of time.

Mellanby (1933) showed experimentally that the larvae of *Xenopsylla cheopis*

are easily killed by drying but the pupae are resistant to dry environmental conditions.

It is only in the adult stage that the flea is parasitic. The animals which harbour fleas are those that provide themselves with some nest or den. The flea is a temporary parasite and uses its host as a source of food and frequently leaves it between feeds.

The average flea is able to jump about 3 inches from the ground when gorged, and about 4 inches when starved. It is also capable of walking up a vertical sheet of glass for about 8 inches after which it falls back to the ground. Horizontally it is unable to cross a ditch 6 inches wide but it can remain afloat on water for some hours.

Fleas of either sex live exclusively on blood which is an absolute necessity for their own nutrition, also for the nutrition of the ovum. If fleas are unable to obtain the blood of the proper animal on which they are usually found, they may attack other animals. Under normal circumstances the rat flea will always be able to find a rat to return to but where the number of rodents has been practically decreased which happens during an epizootic of plague among rats, it is possible that the flea may be driven by hunger to attack man. Even it will not refuse to bite caterpillars. This explains the occurrence of cat and dog-fleas on rats, and rat-fleas biting man.

A female flea cannot be induced to lay eggs without a blood meal and it is believed that like mosquitoes and other blood sucking insects, blood is an important element for the formation of the egg.

Fleas are very sensitive to light and also to air currents. They always hide under dark objects, and when a number of fleas are blown upon they at once become very agitated.

Oviposition takes place within 24 hours of copulation but copulation, according to Strickland (1914), does not take place in *N. fasciatus* unless the flea has fed on rat's blood. The effect of rat's blood is rather stimulating than nutritive. This flea bites man as readily as it bites the rat. Hirst (1926) made similar observations in regard to egg laying by *Xenopsylla astia*.

The duration of the life not only of the adult flea but also of its active stages is influenced greatly by the two important factors, temperature and humidity. An increase of humidity and also of temperature has a tendency to retard the growth of all these stages.

Under the most favourable conditions Bacot succeeded in keeping fleas alive for ninety-five days. During summer in Calcutta *astia* and *cheopis* do not usually survive more than approximately 4 weeks even though they have opportunities for feeding on rat's blood regularly.

In unfed fleas high temperatures and low humidities tend to shorten life and, conversely, low temperatures and high humidities with a possible optimum of 99 per cent, tend to prolong it. Webster and Chitre (1930) found under natural conditions in the Bombay Presidency that during hot weather the majority of fleas died off between 2 and 4 days, a few living as long as a week. In Java, on the other hand, Swellengrebel (1913) observed that when *cheopis* were not given any

feed at all since their emergence, almost all of them died within 7 days and only one individual survived till the 20th day.

Feeding, on the other hand, has a marked influence on the life of the flea. When fed once before starvation, *X. cheopis* lives longer than unfed fleas and when they are kept with the host for 7 days before starvation, they live longer. Females live considerably longer than males (Hirst, 1926; Webster and Chitre, 1930; Leeson, 1936).

Although, according to Bacot and Martin (1924), and Hirst (1926), the duration of life of *X. cheopis* is directly proportional to saturation deficiency, Leeson (1932, 1936) pointed out that the life of the flea is determined mainly by temperature and that the effect of humidity, though definite, is relatively slight. The adult flea normally shows a remarkable resistance to low humidity. Humidity no doubt plays an important part in directly influencing the production of offspring of fleas and when it is below a certain figure, no offspring is produced (Buxton, 1938).

In East Africa Hopkins (1935) found a temperature of approximately 20°C and 100 per cent relative humidity the most suitable not only for the survival of adult *X. cheopis* but also for hatching of eggs and production of a further generation of adults. At this temperature and humidity the whole pre-adult life of *X. brasiliensis* and *X. cheopis* was found on an average 8-9 weeks, the lowest being 6 weeks.

Disease Relationship.

The following diseases are carried by fleas. (a) Plague, bubonic in man and epizootic in rats; (b) Rat trypanosomiasis (*T. lewisi*); (c) Cat and dog tapeworms; (d) Murine typhus; (e) Canine and infantile leishmaniasis; and (f) Chiggerosis (by fleas belonging to the family Sarcopsyllidae).

Rat trypanosomiasis. *T. lewisi* is transmitted by fleas parasitic on the rat. Infection in rats can take place by eating infected fleas but commonly it is effected by way of the rat's mouth, the rat licking from its fur or skin the moist faeces of infective fleas containing the final propagative form of the cycle. The trypanosome does not penetrate into the salivary glands of the flea but is confined during its whole development to the digestive tract.

Murine typhus. It is a disease of the typhus group occurring in many parts of the world and the causative organism is *Rickettsia muricola* (*moo'sari*). It is normally transmitted by a rat louse of the genus *Polyplax*, possibly also by the tropical rat mite, *Liponyssus bacoti* and by different types of fleas. The flea most concerned is *X. cheopis*. What appears to be the same organism has been recovered from wild rodents in several parts of the world. The faeces of the infected fleas is infective.

Cat and dog tapeworms. Fleas, particularly cat and dog-fleas, act as the intermediate hosts of the developmental stages (cysticercoid stage) of certain cat and dog tapeworms (*Dipylidium caninum*). Cats and dogs become infected by the ingestion of infected fleas. It is not uncommon to find similar infection in man due to the accidental ingestion of fleas carrying the cysticercoids. The fleas may also serve as intermediate hosts of *Hymenolepis diminuta*.

Canine and infantile leishmaniasis. This disease has not yet been reported from India. Dog-flea was in the past thought to be the vector. Ticks have also been suggested. More recent work indicates that species of *Phlebotomus* are concerned in the spread of this disease which occurs in the Mediterranean littoral, also in China.

In addition to the various diseases carried by fleas, they are undoubtedly obnoxious pests. Their bite, though imperceptible at the time, is very irritating afterwards. A local spot appears; this quickly becomes larger, ulceration very commonly follows scratching. The mark of a flea bite lasts for a long time.

Examination of Fleas.

For mounting fleas freshly collected specimens should be left in 10 per cent caustic potash solution for 24 hours. As far as possible boiling in potash solution should be avoided. It may be necessary to apply gentle heat to hasten the process. In that case sand or water bath should be used. After such treatment all traces of the alkali should be removed first by washing in running water and thereafter by placing them in a 2 per cent solution of glacial acetic acid for 2 hours. The fleas are then transferred to carbolic acid and left standing till the last trace of water has been removed and the insects appear more or less transparent. If necessary, the specimens may be left in carbolic acid for one or two days. The fleas are next transferred to clove oil for 10 minutes and then removed on a slide and after proper orientation mounted in canada balsam.

The carbolic acid method is quite satisfactory when one has to examine a very large number of fleas at a time. The specimens after treatment in carbolic acid are arranged on a slide and covered with cover glass, the space between the cover glass and the slide being filled up with carbolic acid.

In place of carbolic acid some prefer dehydration in ascending grades of alcohol till absolute alcohol is reached. The final process of dehydration is accomplished by treating in alcohol-xylol, xylol, and clove oil. Fine hairs are much better preserved in alcohol than in carbolic acid.

It is necessary to point out that fleas should always be picked up by holding one of the legs with forceps. If the abdomen is unduly pressed, whether at the time of collection or during the process of mounting, the receptaculum seminis is likely to be dislodged from its proper position. This may render the identification difficult.

Specimens which have been kept in carbolic acid for a long time are unsuitable for identification. They can be rendered suitable by immersing in 70 per cent alcohol and washing in water before using caustic potash solution.

Classification. Fleas are grouped into a number of families and genera. A detailed classification is, however, not necessary.

The order Siphonaptera is divided into the following three families: (a) Pulicidae or the true fleas, (b) Sarcopsyllidae or chigger fleas, and (c) Ceratopsyllidae or bat fleas. The last named family is unimportant.

The difference between the two families Pulicidae and Sarcopsyllidae is given below:

Fam. Pulicidae: (i) the head is conical. (ii) The three thoracic segments

are distinct and not foreshortened or telescoped into one another. (iii) The coxae and femorae of all the legs are equally well developed.

Fam. Sarcopsyllidae: (i) The head is angulated. (ii) The three thoracic segments are foreshortened. (iii) The coxae and femorae of the first two pairs of legs are very thin.

The family Pulicidae includes all the common fleas found on rats, cats, dogs and other domestic animals. It also contains those that bite man. Sarcopsyllidae, on the other hand, contains the chigger fleas and others found on domestic animals like fowls, pigs, etc. and sometimes also as fixed parasites.

Family PULICIDAE

The following represents the important genera found on rats.

1. Eyes present.

(a) No comb on either pronotum or genu.

Pulex, *Xenopsylla*.

(b) Comb on pronotum only.

Hoplopsyllus, *Nosopsyllus* (*Ceratophyllus*), *Stivalius*.

(c) Comb on pronotum, also on genu.

Ctenocephalides.

(d) Pygidium strongly convex and freely projecting behind.

Pygiopsylla.

2. Eyes absent.

(a) Teeth or comb on head and pronotum present; abdominal terga without comb; bristles on hind border of tibiae arranged in a close-set row.

Leptopsylla (*Ctenopsylla*).

(b) Teeth or comb on head and pronotum present; abdominal terga (one or more) with comb.

Hystriopsylla.

(c) Teeth or comb on head and pronotum; last tarsal segment of first and second legs with four lateral bristles; 3 antipygidial bristles on either side.

Ctenophthalmus.

Genus *Hoplopsyllus*.

It is distinguished from *Nosopsyllus* (*Ceratophyllus*) from the segmentation of the third segment of the antenna and the antipygidial bristles. In the former the club of the antenna is incompletely segmented and only one antipygidial bristle is present on each side. In the latter, antenna is completely segmented and there are more than one antipygidial bristle on each side.

It is distributed in N. America (California) where one species, *H. anomalus* Baker, is suspected of carrying plague. The comb of the prothorax consists of 8-10 spines. It is found on ground squirrels and is accidentally found on rats.

Genus *Nosopsyllus*.

This genus has recently been separated from *Ceratophyllus* which is restricted to birds and fowls (Jordan, 1933).

The genus *Nosopsyllus* is large and widespread. *N. fasciatus* Bosc. is the common rat-flea of temperate climate and is a dangerous carrier of plague in

Europe. The distribution of this species in India is limited to the hills. In India *N. nilgiriensis* Jord. and Roths. is fairly common in the Nilgiri Hills. In the Punjab *N. punjabiensis* J. and R. occurs in many places but only during the winter months; this species was formerly identified as *N. fasciatus*. *N. simla* is found in N. W. India. *N. (Ceratophyllus) anisus* Roths. is confined to Japan and Manchuria.

Genus *Stivalius*.

Resembles *Nosopsyllus (Ceratophyllus)* but larger in size and the eyes much reduced; also a larger number of bristles on the head.

St. (Pygiopsylla) ahalae Roths. occurs in South India in the hills.

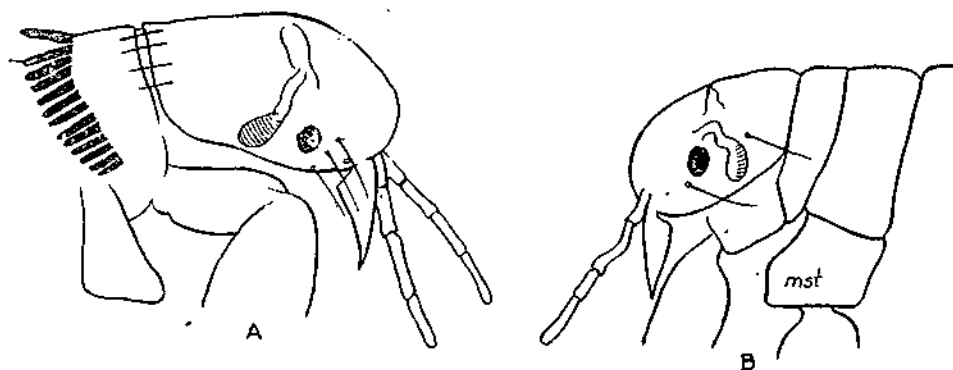


Fig. 117
A. *Nosopsyllus (Ceratophyllus) fasciatus*.

B. *Pulex irritans*.
mst., mesosternum.

Genus *Ctenocephalides (Ctenocephalus)*.

This genus has been erected by Stiles and Collins (1930) to replace the pre-occupied genus *Ctenocephalus*.

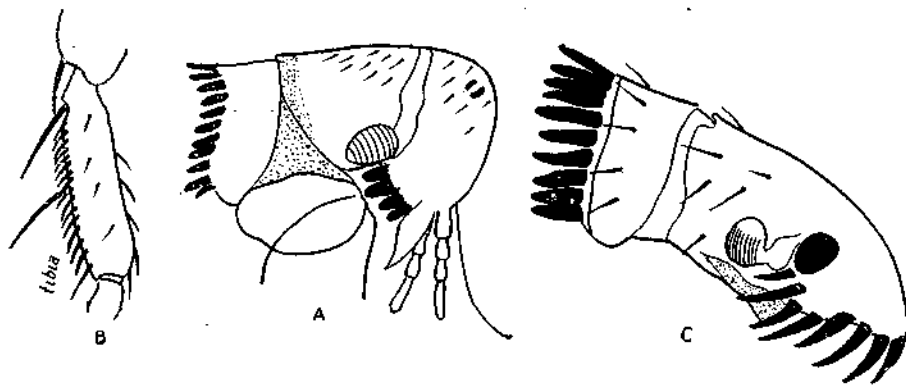


Fig. 118.
A. *Leptopsylla musculi*.
B. Tibia of *Leptopsylla musculi*.
C. *Ctenocephalides felis* var. *orientis*.

These are principally the dog and cat-fleas and are distributed all over the globe. They are known to attack rats and sometimes even man. In India this

genus is represented by *C. canis* Curtis, *C. felis* Bouche and *C. felis* subsp. *orientis* Jordan. These three are very closely related to one another and the subspecies *orientis* is undoubtedly intermediate between *felis* and *canis*. *C. canis* is confined to the hilly regions in India and is very scarce in the plains. In India the subspecies *orientis* appears to be the commonest flea of the genus *Ctenocephalus* and replaces *C. canis*. Its chief host is the dog but it is also found on cat, goat, rat and squirrel.

Both cat and dog-fleas are capable of transmitting plague under laboratory conditions but it is doubtful if they ever play such a rôle under natural conditions.

Key to the genus *Ctenocephalides*.

(After Sharif, 1930)

- I. Anteriormost genal spines much smaller than posterior ones. *canis*.
- II. Anteriormost genal spines nearly as strong as posterior ones.
 - a. Frons elongate and pointed at the anterior end; female without a row of short hairs posterior to antennal groove; *felis* forma *typica*.
 - b. Frons short and broadly rounded anteriorly; female with a row of 1-8 short hairs posterior to antennal groove; *felis* subsp. *orientis*.

Genus *Pygiopsylla*.

In many respects fleas of this genus resemble those of *Nosopsyllus* from which, however, they can be easily differentiated by the characteristic convex pygidium. *P. hilli* is common in Australia on rats. In Java *P. ahalaë* Roths. is suspected of playing some part in the transmission of plague.

Genus *Leptopsylla* (*Ctenopsylla*).

This genus is represented in India by two species, *L. segnis* Schönh. (*Ctenopsylla musculi* Düge) and *L. himalaica* Roths. They are easily differentiated by the number of spines on the genal comb and on the anterior angle of the head. In *segnis* there are 4 teeth on the genal comb and there are two short spines close to the anterior angle of the head. In *himalaica* the genal comb consists of only 2 spines and there are no spines on the anterior angle of the head.

While *L. segnis* is found principally in the plains of India, *L. himalaica* occurs in the hills. Both are mouse-fleas. *L. segnis* is cosmopolitan and may occasionally be found on rats. The latter has been suspected of playing a minor rôle in the transmission of plague.

Genus *Hystriopsylla*.

H. talapae is found on moles and shrews in Great Britain and is only accidentally found on rats.

Genus *Ctenophthalmus*.

They are widely distributed in Europe, Russia, South Africa and America. *Ct. agyrtes* Hell are found on brown rats, field mice, and bank voles in England and also Western and Central Europe. They seldom bite man and are chiefly implicated in the transmission of plague from rodent to rodent.

Difference between the two genera *Xenopsylla* and *Pulex*.

In *Pulex* the sternite of the mesothorax is very narrow, without any internal rod-like incrassation or thickening from the insertion of the coxa upwards. In *Xenopsylla* the sternite of the mesothorax is wider and contains a strong internal chitinous rod which runs from the insertion of the coxa vertically upwards to the upper border of the mesosternum. *Pulex* is essentially a human flea, and *Xenopsylla* a rat-flea.

Genus *Pulex*.

The genus contains one species only, *P. irritans*, which is the human flea and is practically cosmopolitan. It also bites other animals including the rat. In the tropical countries of the eastern hemisphere *P. irritans* occurs only in the hills, though it is found in North, South and Eastern Africa. In Europe and Central Asia it is essentially a parasite of man and is not found on rat or other animals.

In *P. irritans* the bristle situated in front of the eye is absent and is replaced by one below the eye. The hind coxa bears a number of hairs situated on the inner surface of the posterior portion.

Genus *Xenopsylla*.

This genus is represented by a large number of species. It is primarily a rodent flea and is widely distributed. At least one species, *X. cheopis*, is principally concerned in the transmission of plague. Rothschild in 1911 separated the three species of fleas of the genus *Xenopsylla* found on rats. These are *astia*, *cheopis* and *brasiliensis*. In addition to these two rare species, *sewelli* and *hussaini*, both taken from *Gerbillus indicus* have been reported by Sharif (1930) from India.

X. cheopis Roths. It is widely distributed in the tropics and is the principal plague-flea in India, Ceylon, West Africa and Hongkong. It is thought to be originally a native of tropical Africa. It is very widely prevalent in the plains and also in the hilly regions in India.

In the male *cheopis* the antipygidial bristle is situated on a short pedestal, which is placed at some distance from the apical edge of the 7th segment. The ninth sternite, which usually projects but slightly from the interior of the eighth segment, is widened towards the apex, having more or less the shape of a club.

In females the "tail" of the receptaculum seminis is very long and near the constriction it is a little wider than the head. The abdominal segments III to VI bear ventrally on each side a row of three or four, rarely five, bristles and the eighth segment has less than 30 bristles, usually 20 to 25.

X. brasiliensis Baker. It is found in India and Africa and has recently been introduced into South America from Africa. Its distribution in India is very restricted.

In the male the antipygidial bristle is placed on a long pedestal, which projects beyond the apex of the 7th segment. In the female the "head" of the receptaculum seminis is very much wider than the "tail". The abdominal segments III to VI bear ventrally on each side a row of 4 bristles, and the eighth segment has on the outer surface less than 20 bristles.

X. astia Roths. Like *cheopis* it is prevalent all over India. Before the separation of the three species by Rothschild, it was confused with *cheopis*. Outside India it is found in Burma, Ceylon, Java and Mesopotamia. *X. nubicus* represents *astia* in Africa.

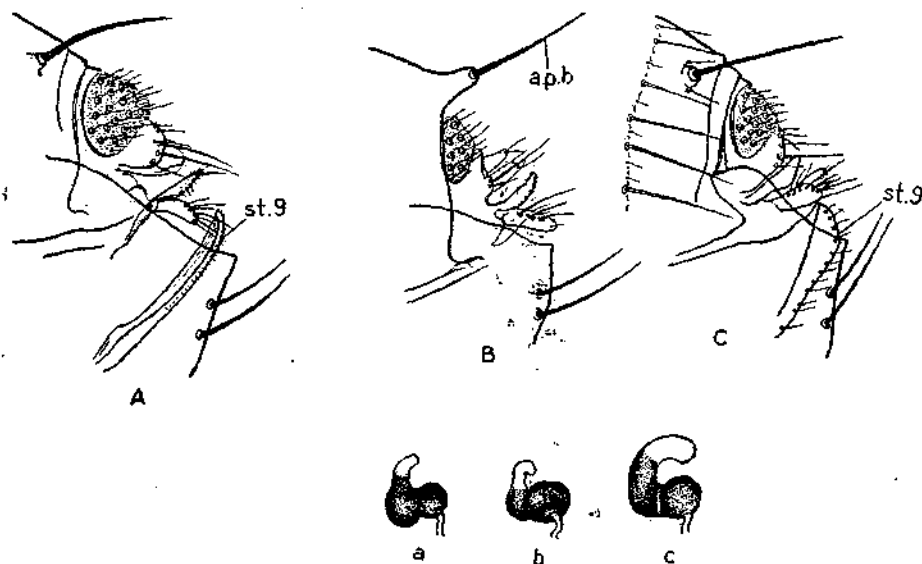


Fig. 119

A. *Xenopsylla astia*, male. B. *Xenopsylla brasiliensis*, male. C. *Xenopsylla cheopis*, male.
a. Receptaculum seminis of *X. astia*; b. Receptaculum seminis of *X. brasiliensis*.
c. *X. cheopis*. ap.b, antipygidial bristle; st. 9, 9th sternite.

The antipygidial bristle in male *astia* is similar to that of *cheopis*. The ninth sternite is characteristic. This sternite instead of being club-shaped has the appearance of a ribbon viewed from a point on its edge, which is due to the ventral margin being strongly chitinised, whereas the sides and the upper margin are very thin and transparent.

In the female the "tail" of the receptaculum seminis is so strongly widened near the constriction that it is here very much wider than the "head"; the "tail" moreover is shorter than in *X. cheopis*. The abdominal segments III to VI bear ventrally a row of 7 or 8 bristles on each side, and segment VIII has more than 30 bristles on the outer surface..

Key to the identification of the species of *Xenopsylla* (Sinton, 1925)

Male.

1. With antipygidial bristle on a long pedestal, which projects beyond the apex of the 7th segment. *brasiliensis*.
2. With antipygidial bristle on a short pedestal, which does not project beyond the apex of the 7th segment.
 - (a) IXth sternite club-shaped. *cheopis*.
 - (b) IXth sternite ribbon-shaped. *astia*.

Female.

1. The "head" of the receptaculum seminis very much wider than the "tail". *brasiliensis*.
2. The "tail" near the constriction is wider than the "head".
 - (a) With the "tail" near the constriction very much wider than the "head"; the "tail" comparatively short. *astia*.
 - (b) With the "tail" near the constriction distinctly wider than the "head"; the "tail" comparatively long. *cheopis*.

The following genera of fleas belonging to family Pulicidae are found on rats in India.

1. *Pulex*, 2. *Xenopsylla*, 3. *Leptopsylla*, 4. *Ctenocephalides*, 5. *Nosopsyllus* and 6. *Stivalius*.

The distribution of *Xenopsylla* in relation to plague.

After studying the distribution of the different species of fleas on rats in different parts of Colombo, Hirst (1913) made the significant observation that *astia* is as a rule the only rat-flea present and that *cheopis* has a patchy distribution and is chiefly found in the plague infected areas during the presence of an epidemic. From this he propounded the hypothesis that *cheopis* is the principal plague-flea and that the relative prevalence of the two species on rats would be one of the most important dominating factors in the outbreak of an epidemic. It may be pointed out that in 1913 Hirst for the first time reported that the rat-fleas of Colombo were *X. astia* and not *X. cheopis*. The Indian Plague Commission had not made any distinction between those two species.

Cragg (1921) first initiated a survey of the specific flea-indices on rats from different parts of India. He came to certain well defined conclusions:

- (1) *X. brasiliensis* is confined to Peninsular India, i.e., Bombay and Madras Presidencies, also in Central India, this species being totally absent in Bengal, Assam, Burma, United Provinces, and North Western Frontier Provinces.
- (2) *X. astia* and *X. cheopis* occur all over India, equally in the peninsular and extra-peninsular portions; their prevalence cannot be correlated with climatic conditions, taking for example two extremes of climate, e.g., Punjab and Bengal, where both species are present in considerable numbers. Regarding their relative proportions *cheopis* is the commoner flea in the Punjab, while *astia* is more common on the east coast where the climate is tropical throughout the year and the humidity is always very high, e.g., in Bengal, Assam and Madras.

A large number of surveys have subsequently been carried out and it has been found that the distribution of *cheopis* in relation to plague in India is, in a broad sense, fairly constant. Where the *cheopis* index is high, plague almost invariably occurs and where it is the sole species, a high epidemic rate is common. Where *astia* is in excess of *cheopis*, such places may be (1) either plague-free, (2) a small outbreak may occur, (3) or in certain localities this flea may also play the same rôle as *cheopis*. *X. brasiliensis*, on the other hand, is thought unlikely to be significant in the epidemiology of this disease.

According to King and Pandit (1931) *astia* can act as a vector under certain

conditions without *cheopis* but the epidemics are small and are not carried over to the off season. Webster and Chitre (1930) found that *X. cheopis* are able to thrive at all periods whereas the hot weather is the least favourable for *brasiliensis*.

It has been established that when outbreaks of plague occur in parts of different countries of the world, they are always associated with *X. cheopis*. This flea generally follows the line of grain and cotton trade not only in inland places but also from one port to another.

The mechanism of transmission of plague by fleas.

Ogata (1897) and Simond (1898) were the first to demonstrate the connection between plague and fleas. It was conclusively proved by the Indian Plague Commission that plague will not spread in the absence of fleas. The exact method of transmission was discovered by Bacot and Martin (1914). The proventriculus of fleas is provided internally with chitinous processes which project into its lumen in such a manner that when the muscles surrounding the proventriculus contract, the entrance from the stomach is effectively blocked, whereby the regurgitation of blood from the stomach is prevented.

It has been estimated by the Indian Plague Commission that the blood of a rat suffering from plague contains more than 100,000,000 bacilli per c.cm., and that a flea's stomach can hold 0.05 c.cm. of blood containing approximately 5,000 bacilli.

The plague bacilli multiply very rapidly inside the stomach and proventriculus with the result that the lumen of the proventriculus is completely occluded especially in its anterior part. Such a flea is known as a "blocked" flea. The blocking may even extend to within some distance of the oesophagus. The flea soon becomes hungry and is unable to fill its stomach although it makes repeated attempts to feed with the result that the blood drawn in the pharynx cannot pass the blocked zone and is quickly regurgitated back into the blood of the healthy host. The fresh blood which is thus regurgitated has already become infected by coming in contact with the almost pure culture of plague bacilli in the anterior region of the blocked area. As a "blocked" flea is extremely hungry, it will readily bite any animal it happens to come across.

The fate of an obstructed flea depends on the atmospheric conditions; in the dry tropical heat, the fleas are quickly killed, but in the presence of a cool and moist atmosphere, obstructed fleas may remain alive and are capable of conveying the infection for several weeks.

The relative transmitting power of different species of *Xenopsylla*.

The difference in the power of transmission of plague in the laboratory by different species of *Xenopsylla* was demonstrated by Hirst (1923) in Colombo, and Taylor and Chitre (1923) in Bombay. In a series of cage transmission experiments with *astia*, Hirst failed to transmit plague from rat to rat or between mice, whereas with *cheopis* he was successful in several instances. On the other hand, Taylor and Chitre thought that *astia* was capable of playing some part in the spread of epizootic plague, though when compared with *cheopis*, it was a less efficient vector.

The difference in the two results has, however, been explained by the difference in the climatic conditions of the two places. Goyle (1928) also found *astia* a poor transmitter compared with *cheopis*. Similar results were also experienced by Webster and Chitre (1930) who found that *astia* was a much less regular transmitter than either *cheopis* or *brasiliensis*.

Relative biting propensities of *Xenopsylla*.

From the researches carried out by Hirst (1923) it became apparent that different species of *Xenopsylla* exhibited varying propensities for biting man. It has been stated that in Colombo *X. astia* does not readily bite at temperatures above 80°F., whereas Cragg (1921) had found in Agra that at temperatures both above and below 80°F., *X. astia* would feed on man, though not readily. Taylor and Chitre (1923) noticed that between 76°F and 84°F *cheopis* took feed readily whereas *astia* either fed almost at once or not at all. On the other hand in laboratory biting experiments, Webster and Chitre (1930) found that fleas may or may not readily accept a feed even in the presence of a rat, a guinea pig and man. In the presence of a rat, however, the fleas are not readily attracted to the human skin, but when starved, both sexes of the three species feed quite readily on human blood. It is always noticed in Calcutta that while handling fleas in kerosene tins containing rats, both *astia* and *cheopis* show a marked indifference towards man even in spite of an enormous number being present. They will freely crawl on the skin without making any attempt to bite. The fleas will maintain the same attitude even after 48 hours' starvation.

Flea Index.

The method of survey by counting fleas found on rats is imperfect as it makes no allowance for the floating population of fleas at large in rat nests. It is generally assumed that the unknown proportion of fleas which exist on the rat's body is approximately constant. However, in the absence of any better method of studying the population of fleas on rats, the present method is to be depended upon.

The flea-index represents the population of fleas on a single rat. This is determined by catching rats and collecting the fleas after killing both the rat and the fleas with chloroform. The specific flea-index signifies the population of different species of fleas on a rat and this can only be determined after the fleas have been identified.

In collecting fleas from rats the possibility of plague infection in the latter should be borne in mind. It is therefore essential that all those who have to work in plague areas must be protected by prophylactic vaccination. The procedure of collection of fleas from rats is as follows.:

Traps with suitable baits are set at night especially in granaries. A suitable trap in which the rats can be caught alive should be chosen.

In order to prevent the fleas from escaping, the cage with the trapped rat is placed inside a cloth bag, and the two ends of the bag are tied with a string. After the bags have been brought to the laboratory, they are placed in a chamber which will allow the killing of the rat without the latter being taken out of the

cage. When the rats and the fleas have all been killed, the bag is removed from the chamber and the rat cage is taken out of the cloth bag. The dead rat is placed on a white sheet of paper, its body is combed with a steel brush and the fleas will drop on the paper. The axillae, groins and neck are the parts which are generally infested. The bag should now be turned inside out and examined. After the collection of fleas from individual rats, the insects are preserved in spirit, and the tubes are properly numbered. The dead rat is placed in a strong solution of lysol till it is destroyed by burning.

Specific flea-index in relation to the epidemiology of plague.

In view of the fact that under experimental conditions both *cheopis* and *brasiliensis* have been found to be better transmitters of plague than *astia*, it is apparent that where a specifically pure flea population is concerned, a proportionately much higher *astia* index is required for the continuance of epizootic plague. The exact numerical value of the different species cannot be laid down. Webster (1930) believes that with an *astia* index of 7, the epizootic can restart, this figure being greatly reduced during the post-epidemic period. The relative importance of different fleas as transmitters of plague has been assessed by Webster as 1: 0.3: 1.7 respectively in *cheopis*, *astia* and *brasiliensis*. In the case of *cheopis*, Hirst thinks that even a flea-index of 1 can start an epidemic.

Principal plague fleas.

For the purpose of determining the vector which is locally responsible for the carriage of plague bacilli from rat to man, it is not possible to demonstrate the presence of the bacilli in the proventriculus and midgut of the flea. The fleas which are found on rats in sufficient numbers during epizootic and epidemic outbreaks and which come into intimate contact with man are always suspected of acting as the vectors of this disease. The following list gives a fair idea of the principal plague fleas in different parts of the world.

India and Ceylon:	<i>X. cheopis</i> and possibly <i>X. astia</i> .
Japan	: <i>N. (Ceratomyx) anisus</i> .
Australia	: <i>N. (Ceratomyx) fasciatus</i> and possibly <i>Pygiopsylla ahalae</i> .
Great Britain	: <i>N. fasciatus</i> .
Europe	: <i>N. fasciatus</i> and possibly <i>Ctenophthalmus agyrtes</i> .
Java	: <i>X. cheopis</i> and possibly <i>Pygiopsylla ahalae</i> .
West Africa	: <i>X. cheopis</i> .
North America	: Possibly <i>Hoplopyllus anomalus</i> .
Ports in Europe,	<i>X. nubius</i> .
America, Africa,	<i>X. cheopis</i> .
India, and Far	
East.	

Rearing fleas.

(1) The fleas are kept with a mouse in a large glass jar. The mouse is provided with autoclaved oats, a small quantity added daily and drinking water. The

water is contained in a specimen tube closed with a cork bored to take a small length of glass tubing; the end of the tubing, which projects about $1\frac{1}{2}$ inch has been previously rounded off in a Bunsen flame, so that the opening is less than half its original diameter. The whole is then suspended by a wire, mouth downwards, inside the jar. The jar is covered with a fine muslin cover over which is fitted a perforated zinc cap.

When fleas are required the debris is tipped into one side of a large battery jar: the other side is shaded; the adult fleas hop into the shade and are collected with the aid of a suction pump attached to the nearest water tap. (Leeson, 1931).

(2) Fleas may also be bred on a rat in a kerosene tin placed on a trough containing water to which some phenyle is added to guard against ants. The rat is kept in a cage on a bed of coarse sand and bread well soaked in milk to which codliver oil is added, is given once a day. Occasionally green vegetables are also given. All rats do not always harbour fleas on their bodies and therefore it is necessary that in the beginning the rat should be taken out every night and placed in some parts of the building which are well frequented by rats at night. In order to attract other rats some food is placed just outside the cage. In this way fleas will be picked up by the healthy rat. In the day time the rat is always kept in the tin. From the time a rat with living fleas is introduced, the culture becomes ready within 3 to 4 weeks. In this way *astia* and *cheopsis* may be reared separately. During the rainy season in the tropics, the continuous breeding of fleas may be interrupted on account of the development of *Tyroglyphus* mites in the breeding jar. It is therefore necessary to remove the rat with a large number of live fleas to a separate tin containing dry sand. The original tin may be set aside and its fleas in the meantime may be utilised for work.

(3) For oviposition by individual fleas, small glass jars should be selected. If some rough paper folded in a longitudinal manner is placed inside, fleas may be induced to lay eggs in the folds. The paper should be of a dark colour; it then becomes easy to count the number of eggs laid by the flea. The paper with the egg attached to it is cut up into bits and placed on sand inside a wide-mouthed glass phial. Dried rat's or rabbit's faeces and powdered blood are used as food for larvae.

Dissection of fleas.

On account of the presence of tough chitin on the body, dissection of fleas is difficult. The needles must be strong; one must be blunt pointed for steadying and the other sharpened to have a knife edge for cutting. The type of instrument used

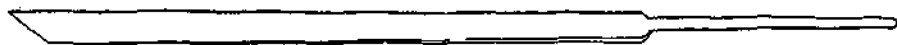


Fig. 120
Special cutting knife for dissection of fleas, bed-bugs and lice.

in this country by barbers for cutting finger nails has been found to be very useful. This is a rectangular steel rod as wide as the handle on which dissecting needles are mounted and has a sloping sharpened edge at one end. This is strong and at the same time the edge is sufficiently sharp for chipping off the chitinous cuticle of fleas.

The legs are removed with the knife-edged needle from the attachment of the coxa with the body. Dissection is done in salt solution on a glass slide. The insect is steadied with a needle by pressing the blunt end against the ventral part of the abdomen. This will push the alimentary canal towards the dorsal side. The abdominal sternites are now cut along the ventral margin. In the same way the cuticle along the dorsal margin of the abdomen is chipped off. The tergites and the sternites of the different abdominal segments are then gradually freed from their tracheal and other attachments and removed with the help of forceps. In this way a large part of the alimentary canal becomes free. Though the salivary glands generally project out from the metathorax some further dissections may be necessary in order to have a good view of these organs.

The other method of exposing the alimentary canal is to steady the insect, and then gradually and carefully push a knife-edged needle between two segments about the middle of the abdomen. The nick is extended and carefully widened. The posterior part of the abdomen is cut transversely and the alimentary canal is exposed by pulling the head anteriorly.

Section cutting.

For cutting sections of fleas double embedding in celloidin and paraffin will be necessary. More simple and better method has been described in connection with the section cutting of ticks.

Anti plague Measures.

Bubonic plague would at once disappear from this earth the very day the intimate contact between rat and man is broken. This, however, will not affect the occurrence of epizootic plague among rats. The essential part in the prevention of plague is the destruction of rats, especially *Rattus rattus*, which generally nests inside the house, and sometimes in refuge dumps adjoining the house.

Godowns should be properly planned both regarding their sites and construction. Legislative measures in order to prevent their construction in the midst of human habitations are necessary. Food grains should not be allowed to be stored on a large scale in unlicensed houses. The godowns must be not only rat-proof but also equipped with arrangements for fumigation. The object of fumigation is not only to kill rats but also insects infesting foodgrains. Store houses should always be located some distance from human habitations. In cases of outbreaks of epizootic plague among rats there is a great tendency on the part of the latter to quickly disperse from their nests.

Rats should be destroyed not only for the reason that they are the principal source of bubonic plague in man, but are also responsible for much economic loss. A full-sized rat can eat and waste about 27 pounds of wheat or about 20 pounds of flour in one month. It has been estimated that 100 rats in India without breeding will consume and waste about 27 maunds of grain a year, and 70,00,000 tons of food grains are consumed by rats in India every year. This would mean that 18 million people could be fed at 1 pound per day for one whole year.

The depredation caused by rats is well known. Doors are frequently knawed for making openings for their ingress and egress; even lead pipes if they stand on

the way may also be attacked. It is amazing how rats can crawl up pipes in order to reach the upper storey from the ground and can also freely travel on ropes of boats when they are anchored in harbours.

It is interesting that mice, though they harbour fleas, seldom die of plague.

Extermination of rats is always a matter of considerable difficulty on account of their habit of hiding in holes in inaccessible places and their capacity to increase their progeny at an enormous rate. The first impregnation may take place within three months after birth and the gestation period is approximately three weeks. Impregnation can be renewed within a few hours of the birth of a litter and the female may have 5 to 6 litters in a year. The number of rats in a litter varies from 6 to 18, and 10 to 14 is very common. The reproductive activity of a rat generally lasts for two years. The descendants of a single female may number over 100 at the end of one year and in course of three years this may reach 2,000,000.

The two most important species of domestic rats are *Rattus rattus* and *R. norvegicus*. The former is popularly known as the black rat and the latter the brown rat.

R. rattus (*R. alexandrinus*)—The tail is often about one and a quarter times as long as the head and body combined; the eyes are large and prominent; the ears are large; muzzle narrow; tail uniformly dark; feet slender, white but sometimes dark. The colour in the Indian species is usually brown and the belly yellowish white. It is widely distributed in India from the sea level to an elevation of at least 8,000 feet. It is a domestic rat and is a good climber.

R. norvegicus. The tail is about 90 per cent the length of head and body. It is a heavy-bodied rat with large, heavy tail which is generally white or distinctly light in the lower half; ears and eyes small, the feet are large, heavy and flesh-coloured; no long piles or bristles on the body (chief characteristics in *Nesokia*). It does not spit or bristle when caged. It is a drain rat and visits house at night. It is also a burrowing rat and a good swimmer.

There are various methods of dealing with rats in the house, the chief of them being (a) trapping, (b) poisoning and (c) fumigation. The plan of action must be systematic and sustained and not a sporadic attempt to merely reduce their number every now and then or during the time of outbreaks of plague.

Trapping. Various types of traps are sold in the market. The "wonder" type which can trap more than one rat at a time is widely used. In dealing with a large colony of rats, traps should never be set unless a very large number are set simultaneously. Under such conditions poisons should better be used.

Poisoning. It must be specially noted that no baiting programme will succeed when there is plenty of waste food scattered in the house. Further, a proper cleaning up should always be done before baiting is resorted to. It is only then that this method of dealing with rats will prove of considerable value.

Poisons should always be handled carefully and also in the matter of their use extraordinary precautions must be exercised. Some of them are deadly poisonous not only to domestic animals but also to human being. They should never be administered by spreading them on bread; there is then the chance of their being scattered by rats at night.

When it is intended to kill rats by using poison, the latter should be used on an extensive scale being distributed not only in every part of the house but also in the garden. If possible, the flavouring agents and the meal with which the poison is prepared should be changed, so that if the rats suspect one kind of bait, they will take the other.

Rats are particularly attracted to tallow and next to it, lard. They are also fond of fish especially shrimp. The fish must of course be cooked thoroughly and then broken up for mixing with the poison. Cooked meat is also a good bait.

For preparing the meal either flour, biscuit, powdered gram, powdered perched rice, meat, liver, fish, or cheese may be employed. The poisoned meal may be distributed in the form of cakes or balls. All poisoned meals must be coloured with a dye; Prussian blue, lamp black or chrome green may be used.

The poisons commonly employed are (1) arsenic, (2) phosphorus, (3) strychnine, (4) barium carbonate, and (5) squill.

Among them arsenic and barium carbonate are tasteless and odourless. Though phosphorus has a peculiar smell, yet rats and mice readily take it. Strychnine is bitter and it has got to be mixed with an adequate amount of sugar. Squill is the bulb of the sea leek, *Scilla maritima*, which grows along the shores of the Mediterranean ocean. The fresh bulb may be chopped up or it is dried and powdered. 1 grain of powder is equivalent to 5 grains of the bulb. 1 part of the powder to 16 parts of the meal will form an adequate proportion.

In the case of arsenic and barium carbonate, 1 oz of each in 1 pound of the meal will form an effective poisoned bait. To kill a big-sized rat about $1\frac{1}{2}$ grains of barium carbonate is necessary and when the mixed meal is distributed in the form of balls, each ball should contain this amount. After taking barium the animals feel very thirsty and they run about and therefore seldom die in the hole. This is a great advantage of using barium. Further it is only fatal to man when taken a dose of at least 60 grains. There is therefore a good margin between the fatal dose for a rat and for a man. It is on account of these advantages that it is recommended for domestic use. The death of the rat occurs within a few hours to 2 to 3 days.

The chemically pure carbonate is expensive and the commercial form or the one precipitated by sodium bicarbonate should be used.

While barium is of particular value on account of its harmlessness as a rat poison which can be used in the household systematically throughout the year, both phosphorus and arsenic are the most effective for use in granaries, particularly during an outbreak of plague. Danger to human life can be obviated by care in handling and distribution.

Mouse virus: Living organisms have been tried against rats and mice. These are, in addition to the mouse typhoid bacilli, *B. enteriditis* of Gaertner, the paratyphoid bacillus and the hog-cholera bacillus. The employment of the last is attended with danger to man. While all these have proved successful in laboratory experiments, they have not shown their usefulness in the field.

Fumigation. Rats can be easily killed by burning sulphur or by passing hydrocyanic acid gas in the holes. All holes through which the gas escapes should be

closed with earth. Sulphur should be mixed with some nitre and tallow. The mixture is soaked in a piece of rag which is wound round a stick. Special apparatus has to be used for the cyanide or cyanogen gas. Both are extremely poisonous and need careful attention during handling.

As D.D.T. is reputed to possess marked residual effects on insects in general, one may be tempted to use it for spraying rat-holes as an anti-flea and anti-plague measure. As we have succeeded in recovering living fleas from rats forcibly expelled from holes which had been sprayed with a 5 per cent mixture of this insecticide a week previous to the experimental introduction of the rats, we believe that its employment for the purpose stated above will not be fruitful.

Cat and dog-fleas.

At times they are troublesome to man and from these animals they often spread to rats and become potential agents in the spread of epizootic plague. The breeding places of these fleas must be attacked and preferably with pyrethrum spray. Pet animals should be dusted from time to time with a 5 per cent powder of pyrethrum.

Personal prophylaxis.

The following measures are recommended.

(1) The wearing of gum-boots or ordinary boots and putties during the plague season.

(2) Spraying the entire floor of a dwelling house including outhouse, stable, cellar, kitchen, store house and lumber-room, with either (a) kerosene + naphthalene, (b) kerosene + D.D.T., or (c) kerosene + pyrethrum + oil citronella once a day during the plague season.

(3) Prophylactic inoculation of plague vaccine ; the protection lasts for one year.

(4) When a dead rat is noticed in the house, the rat should be handled with forceps and dropped in a pot containing some kerosene. The rat should be brought to the nearest laboratory for post-mortem examination. During the plague season all such deaths in rats must be suspected to be due to plague and intensive spraying must be done in the house. In addition to spraying, anti-rat measures are immediately undertaken. The house should be properly cleaned and tidied up. All cracks in floor and walls should be at once filled up with cement and broken glass. There should be no opening in the door through which rats may enter the house. If necessary the lower 6 inches of the door may be protected with a sheet of galvanised iron. All drainage holes must be covered with wire netting.

Family SARCOPSYLLIDAE.

In this family the mouth parts are directed forwards. The legs are not nearly so well developed as in Pulicidae on account of their parasitic mode of life.

The labial palp consists of three or fewer, very feebly chitinised segments ; the genal edge of the head is in all cases produced downwards into a triangular lobe situated behind the mouth parts. The three segments of the thorax are always shorter than the first abdominal tergite.

Key to the genera of Sarcopsyllidae.

1. Head small and blunted ; hind coxa has a patch of small spines.
Echidnophaga.
2. Head larger and angulated ; patch of small spines on the hind coxa absent.
Tunga.

Genus *Echidnophaga* Olliff.

E. gallinacea Westwood. It is an old world species but has now been introduced into North America and Western Australia. It is widely distributed in the tropics. Its chief host is poultry but it attacks other animals as well. On poultry it is found especially on the naked parts of the head and the neck.

In Texas, *E. gallinacea* not only attack poultry but also children, the latter somewhat severely. Kelsall (1927) reported an interesting case of sweating blood in a human female. A large number of fleas of this species were extracted from little black specks in the neighbourhood of each nipple of the breast.

This species can be easily trapped by letting a guinea pig loose in the infested place and then chloroforming it (Turkhud, 1928).

Fifth tarsal segments in this species have two ventral apical bristles ; hind edge of the head with a lateral lobe.

Genus *Tunga* Jarocki (*Dermatophilus*).

Of the known species of this genus only 2 are found in the old world. *T. caecata* Ender., in which the eyes are vestigial, occur in Brazil on rats. In *T. penetrans* L. the eyes are distinct. This is the flea which causes chiggerosis.

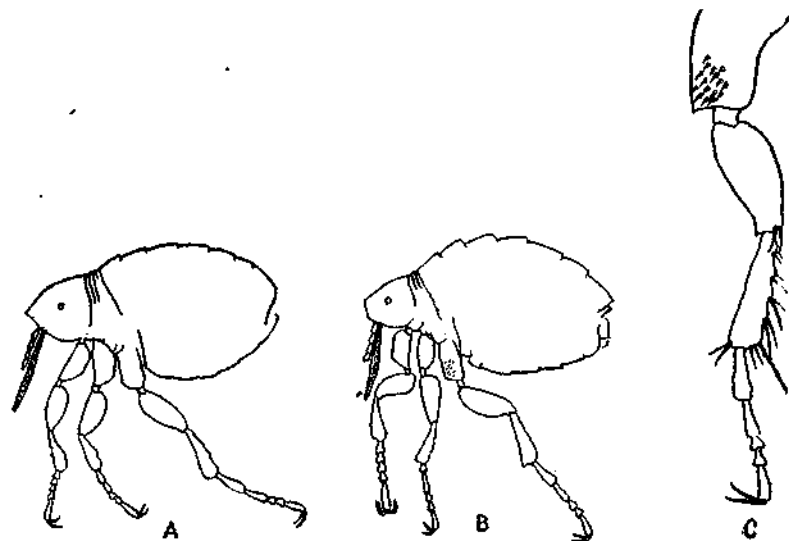


Fig. 121
A. *Tunga penetrans*. B. *E. gallinaceus*. C. Hind leg of *E. gallinaceus*.
(After Turkhud)

T. penetrans Linn. has occasionally been recorded from the western ports of India. This is originally a South American species and has been introduced into

other tropical countries. In Africa it has propagated with astonishing rapidity. In East Africa it is so common that during the Great War it was a source of great annoyance to the British sailors to India. Although it might have been introduced into this country yet it has not been able to gain any foothold. It flourishes best in sandy soil. Its hosts are usually domestic animals from which man becomes infested.

Chiggerosis.

Chiggerosis is caused in man and domestic animals by *T. penetrans*. After fertilisation the female attaches itself firmly to the host. It imbeds itself in the skin generally of the foot and less commonly of the hand. The toes and fingers are affected, also the palm of the hand and the sole of the feet.

In this condition the insect grows from a minute size to that of a pea on account of the distension of the abdomen by the ovaries containing eggs. There is considerable inflammation of the surrounding skin which is raised; the insect lies in a depression in the centre and the terminal segments of the abdomen project outside for the discharge of the eggs. The eggs fall to the ground where further development takes place. After the eggs have been discharged, the female shrivels up and dies.

Treatment is mainly surgical. The insects are first killed with 8% pyrethrum ointment applied over the affected parts for 24 hours and thereafter the dead fleas are removed with forceps.

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Order LEPIDOPTERA (Butterflies and moths)

This order includes the two familiar insects, moths and butterflies. They have a graceful appearance and possess sufficiently distinctive characters by which they can be easily recognised. The following are some of the important characters which comprise this order. The body is densely clothed in hairs and scales. Wings which consist of two pairs are also scaly; they are proportionately of large size and are peculiarly ornamented. The mouth part consists of a long tubular proboscis which is kept coiled up and is extended only during feeding.

The metamorphosis of all *Lepidoptera* is complete. Eggs are laid on leaves of plants on which larvae are also found. The butterfly egg can be easily distinguished from the moth egg. The former is dome-shaped while the latter is rounded. The larva has a distinct head; the three thoracic segments are also distinct. There are three pairs of legs. On the ventral surface of many abdominal segments are present pseudopods or suckerfeet which help the larva in crawling on leaves. The larva lives entirely on leaves of plants and different species have particular attractions for different types of plants. When the larva attains maturity, it spins a cocoon inside which pupation takes place. The pupa is highly chitinised. The abdominal hooks of the pupa assist the imago in escaping from the cocoon.



Fig. 122
Larva of *Lepidoptera*.

The imago is a strictly fluid feeder and lives on nectar of flowers or on vegetable sap. The imaginal life is generally short and many adults do not feed at all.

This order though of great importance to agriculturists, florists and horticulturists is not so to us. It is on rare occasions that they come to the notice of the medical man. There are some caterpillars which are either uniformly hairy or with erect dorsal or lateral tufts. These hairy caterpillars are responsible for a sort of distressing dermatitis which is commonly met with in this country among gardeners during the rainy season. This is caused by the stinging hairs of the caterpillar. The affected part becomes red, is extremely irritable and becomes swollen. In severe cases nervous symptoms are also present. Most observers agree that the injury is inflicted by hairs of a particular kind which are disposed in definite tufts. These hairs are generally hollowed at the base and barbed at the end; the base contains a gland composed of one or two cells. Sometimes the hairs may be blown into the eye and may set up conjunctivitis.

Treatment of such dermatitis consists in the application of lotio calamine or slaked lime mixed with water.

Some caterpillars infest stored grains and they not only live on the grains but make the latter unsuitable for human consumption.

Order HYMENOPTERA

(Ants, bees, wasps, etc.)

This order represents ants, bees and wasps, and if it were not for the pathological effects they cause to man, this would not have come within our purview. In certain ways their activities are beneficial to man, e.g. they produce honey and wax. A large number of them parasitise flies in their early stages and act as their natural enemies.

The following constitutes the chief characters of this order.

(1) Mouth parts are adapted both for biting as well as for sucking liquid food; the mandibles are well developed. (2) Wings may or may not be present but in typical Hymenoptera there are two pairs of small wings. (3) In the female the abdomen ends in an ovipositor which may be modified to form the sting; the sting generally lies concealed in a sheath. (4) Metamorphosis is complete.

Bees, wasps and ants occasionally inflict painful bites to man. These may also attack in swarms. There are records where death of the individual has occurred.

The venom of the honey bee induces a rapid fall of blood pressure and a dilatation of the splanchnic vessels causing haemorrhage in those areas. It also affects the heart. Injected into the human skin it causes a reaction identical with that caused by rattle-snake venom and by histamine. The venom is markedly haemolytic, haemolysis being preceded by a large increase in the volume of erythrocytes. (Essex, Markowitz and Mann, 1930).

The parasitic Hymenoptera are directly responsible for the destruction of Muscoid larvae and pupae and also larvae of Lepidoptera. The parasite grows at the expense of the host.

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Order ORTHOPTERA

(Cockroach, earwigs, stick insects etc.)

Only one insect in this order concerns the medical man and that is the cockroach which has already been dealt with previously.

Order COLEOPTERA

(Beetles)

A very large number of insects are represented in this order which is primarily important to the forester, agriculturist, stockist of grain and householder. They cause extensive damage to trees, garden vegetables and crops. They also cause an enormous loss of stored food grains including dried fruits. Timber, carpets etc. are also attacked by them. The parts adults and larvae of aquatic beetles play in the destruction of mosquito larvae can not be overlooked. This order is characterised by:

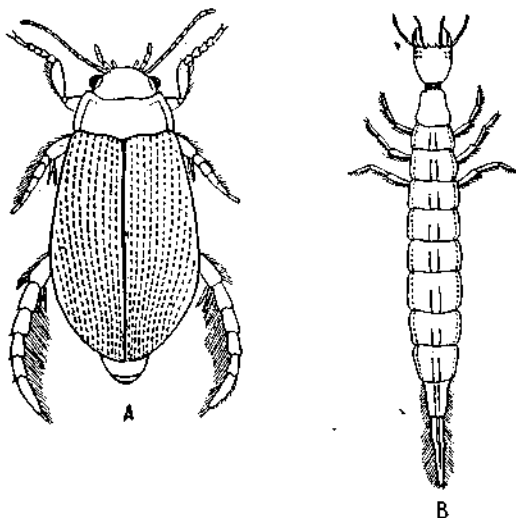


Fig. 123
A & B Adult and larva of *Cybister*
(aquatic beetle).

(a) Strongly built body ; (b) Mouth parts adapted for biting and chewing solid food ; (c) Presence of double pairs of wings ; the anterior pair is the elytra and acts as a protective covering for the membranous hind pair which is used for flight ; (d) Complete metamorphosis.

longer than 72 hours during the winter months in Calcutta. Patton and Cragg (1913) found that the first stage larva when starved does not live more than 2 days.

The growth of population of lice has been calculated by Buxton. The female louse lives 34 days and during that time she does not lay any egg on days 1-2 and 34. As she lays 9 eggs a day, the hypothetical number at the end of 34 days should be 279 which represents the progeny of a single female. As the number of lice generally found on the head or body falls far short of the expected total it is evident that there is always a considerable loss of life, perhaps in all stages of its existence, which tends to keep the population within certain limits.

It has been pointed out by Buxton that long-haired individuals as a rule show a greater infestation rate than those possessing short hairs. On the other hand, Roy and Ghosh (1944) reported the highest count of lice, *i.e.*, 5253 (adult 1434, larvæ, 3819) from a short-haired female head. Another case that has lately come to their notice showed a total of 9020 lice (adult 1670, larva 7350). The last patient had long hairs.

It is by no means true that regular washing, and combing the hair combined with the use of oil will completely prevent louse infestation in women. In such cases the population of lice is generally maintained at a low level.

In nature a disproportion in the distribution of the two sexes is a marked feature. A preponderance of females over males has been noticed.

The activity of lice is greatly dependent upon the temperature of the surroundings. They are very active at 30°C, and at 37°C they are extraordinarily active creatures.

Howlett (1917) has recorded some interesting observations on the temperature reaction of lice. Heat seems to cause them to become greatly excited. He found that removal of lice from the head was greatly facilitated if the comb was previously heated before it was applied. Wigglesworth's studies (1941) conclusively prove that the temperature sense is very well developed in this insect. It can detect even small differences.

Lice are usually killed by 30 minutes submersion in water but nits may survive immersion for 96 hours.

Nuttall found that the colour of lice has a direct bearing on their environments.

Dissemination.

The following factors are concerned in the dissemination of lice. After dissemination, the multiplication of the parasites depends mainly on the neglect of personal sanitation, and the wearing of underclothing for days together.

(1) Close contact with lousy individuals in the household, barracks, camps, underground shelters etc., especially when a large number of individuals have congregated together. Hence infestation spreads rapidly during war, in famine conditions and among refugees.

(2) Indirect contact; *e.g.*, through bedding, clothes, blankets, towels, hats, combs, brushes etc.

(3) Wind may be responsible to a small extent by blowing the nits.

(4) It is thought that lice have a tendency to leave the host when the temperature of the body rises due to high fever. Such migration of head lice under conditions of malarial fever has not been observed by Roy and Ghosh (1944).

Genus *Pediculus*.

This genus is not restricted to man alone but also occurs on monkeys and apes.

P. capitis and *P. corporis* (*humanus*).

As a rule *capitis* occurs on the head, mostly about the occiput and about the ears; *corporis*, on the other hand, is confined to the clothing worn next to the skin, being found especially in the seams on the fibres on which eggs are laid. Nuttall (1917) has collected evidence showing that *corporis* may live upon the body and deposit its eggs upon human hair on the breast and axilla. These are no doubt rare instances of their breeding in unusual situations. It may be pointed out that *P. capitis* occur on the head in all seasons though they are found in large numbers during the winter, whereas *P. corporis* are seldom found in the summer except in very small numbers in countries with either very moist or very dry climate. They are no doubt found at all times of the year in temperate climates.

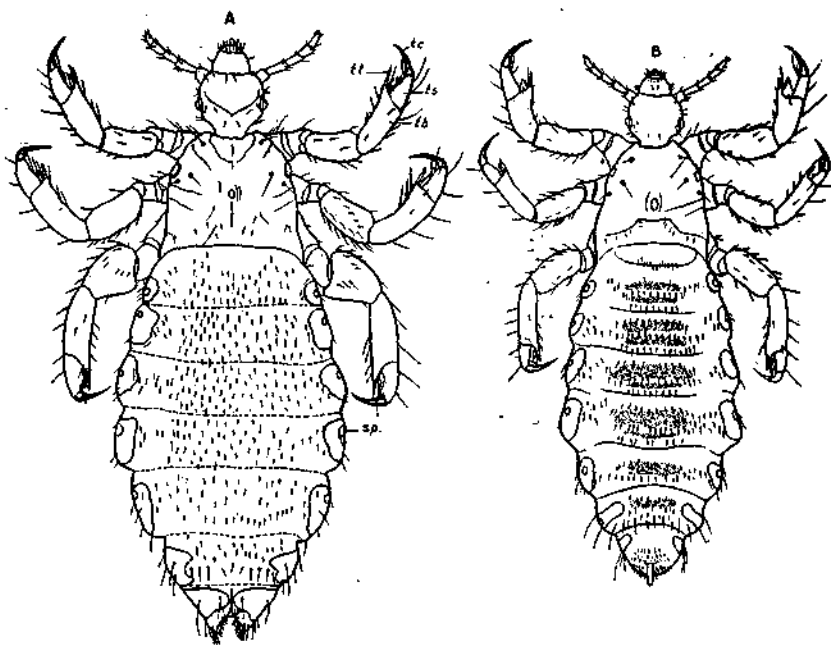


Fig. 131

A. *Pediculus humanus* (*corporis*), female. B. *Pediculus humanus* (*corporis*) male.
(After Keilin and Nuttall).

sp, spiracle; tb, tibia; tc, tarsal claw; ts, tarsus; tt, tibial thumb.

In nature the population of *corporis* increases more rapidly than that of *capitis* for the reason that the females have a relatively greater egg-carrying capacity than the head louse and the number of eggs laid is also greater.

It has been demonstrated that there are no constant differences between the head louse and the body louse and it has also been demonstrated that head lice under certain experimental conditions may acquire all the morphological characters of the body louse after four or more generations (Keilin and Nuttall, 1919). However, certain morphological differences commonly found between them are stated below.

<i>P. capitis</i> .	<i>P. corporis</i> (<i>P. humanus</i> L.)
Size smaller.	Size larger.
Deeply pigmented body.	Pigmentation absent.
Clearly marked indentations between successive abdominal segments (due to chitinous pleural plates).	Abdominal segments are rounded with shallower intersegmental indentations.
Antenna short and broad.	Antenna long and slender, the 3rd joint being relatively broader.
Legs shorter and stouter.	Legs relatively slender and longer than <i>capitis</i> .

Diseases.

Both head and body lice have proved to be arthropod vectors of (1) Relapsing fever ; (2) Typhus fever ; and (3) Trench fever. The first two are very important and both have a wide distribution. In addition to these diseases, lice cause considerable inconvenience to man. They bite especially at night which often interferes with sleep. Their bites are associated with local pigmentation of the skin ; at one time it was known as vagabond's disease. The adenitis met with in the posterior triangle of the neck in children is generally associated with lice. The irritation of the skin due to their bites may result in considerable scratching ; extensive sores may thus appear.

Typhus Fever. Although Nicolle and his co-workers (1909) have demonstrated the transmission of typhus fever from monkey to monkey by means of the bites of infected lice, the causative agent was not known till it was discovered by Da Rocha Lima (1916) both in the peripheral blood of patients suffering from this disease and also from infected lice. These organisms, named *Rickettsia prowazeki*, are found in clusters or pairs within the intestinal cells of the louse and can readily be stained with either Giemsa's or Romanowsky's stain. It was soon made clear that the rickettsia, *R. prowazeki*, connected with typhus and which is pathogenic, is different from the normal rickettsia, *R. pediculi*, harboured by healthy lice, and which is non-pathogenic. In lice *R. prowazeki* undergo multiplication in the epithelial cells of the intestine whereas in the case *R. pediculi*, the parasites are found free in the lumen. Sometimes *R. prowazeki* may even cause the death of the insect host.

In infected lice *R. prowazeki* are passed in large numbers in the faeces and the organisms remain alive in the faeces even when dried. (Arkwright and Bacot, 1923). It has been suggested that the infective agent may be carried by wind. The infection is not, however, carried by the bite, the salivary glands being free from rickettsia, but by the faeces of the insect. This was definitely proved by Atkin and Bacot (1922).

Hereditary infection of the disease does not take place in the louse. Though Sergeant, Foley and Vialette (1921) concluded from experimental observations with nits taken from a typhus patient, that the infection is transmitted to progeny, Nicolle (1921) holds that this occurs when the egg shell is infected with the faeces of infected lice.

Lice can be artificially infected by injecting infecting meal of platelet material through the anus. Under such conditions rickettsia bodies are found in the faeces from the 5th day onwards (Bacot and Segal, 1922).

The rickettsia bodies with which lice become infected, are present in the peripheral blood of the patient up to about the 10th day of the disease. But Mosing (1937) has recently pointed out that as judged by the infection of lice, the virus is not constantly present in the blood even in the first week of the disease ; it may be absent for several hours, even for 2 or 3 days and then again reappear in the peripheral blood stream.

Though both *corporis* and *capitis* can equally convey the disease to monkeys (Goldberger and Anderson 1912), epidemics of the disease are associated rather with the body louse, and generally when the infestation rate among the people and the gross infestation rate on the individual are both high.

Rickettsia infection in louse is determined by teasing the gut and staining the teased-up bits with Giemsa's. Freshly passed faeces may also be examined but the former method is the better.

Relapsing fever. Most of the knowledge acquired in regard to the part played by lice in the spread of relapsing fever has been gathered from the researches carried out by Sergeant and Foley (1908 ; 1910), Nicolle, Blaizot and Conseil (1912), and Nicolle and Blanc (1914).

Mackie (1907) was the first to find body lice infected in nature ; these lice were taken from boys and girls at Nasik who were found to be suffering from relapsing fever. Later, Sergeant and Foley (1908) were able to communicate this disease to monkeys by using infected lice. The life cycle of *Spirochaeta recurrentis*, the causative agent of relapsing fever, within the insect host is different from that of *R. prowazeki*. Within two hours after the spirochaeta have reached the midgut, the motility of the latter completely ceases and they quickly degenerate. Their presence in the body cannot be detected by any means. They reappear, however, between the 8th and the 12th day, an enormous numbers of active spirochaeta being now easily found in the haemocoel of the louse. At first they are small but later they attain the normal size found in the human peripheral blood. The infectivity of lice does not depend on the presence of visible spirochaete. They are most infective about the sixth day just before the spirochaete reappears in the insect. Their infectivity subsequently diminishes. Lice may occasionally be infective as early as the third day and as late as the 15th day after the infective blood meal. The usual method of infection is by crushing the insect at the time it bites which causes the spirochaeta to escape on the human body. Infection cannot be produced by their bites alone.

The best method of demonstrating the spirochaetes in the louse is that described by Riding and Macdowell (1927). The louse is seized with fine forceps, and laid

on its back in a small drop of distilled water on a clean slide. The abdomen is transfixed by a needle lateral to gut, and the hæmocoel fluid is allowed to escape from the puncture, the movements of the legs of the louse assisting the mixing of the hæmocoel fluid and the water on the slide. The fluid can then be examined under the dark ground illumination or stained by Leishman. This method is superior to making smears of crushed lice.

Trench fever like typhus fever is carried by *P. humanus* var. *corporis*. This disease first appeared in the British Expeditionary force in 1915. The causative agent has been thought to be a filterable virus and although the Trench Fever Commission of the American Red Cross Research Committee fixed the responsibility on lice, the exact mechanism of the transfer of infection by the louse was left to the British Committee (1918). They found that the method of infection is by faecal contamination of the wound caused by rubbing and scratching. The louse remains infective for at least 23 days after feeding on the infected patient. The man suffering from trench fever is not infectious to lice for the first three days of his illness; after that he infects them throughout the fever and convalescence.

Breeding of lice.

Both head and body lice may be conveniently raised in a pill box. A hole is punched in the paste board lid and closed by a piece of fine chiffon tied with a silk thread around the rim of the box. The inside is filled with a mass of hair. It is placed inside a leather strap and worn around the wrist (Nuttall, 1917). The pill box may also be conveniently placed between the skin and the socks (Buxton, 1939). Warburton (1910) placed the insects on pieces of cloth in a cotton-plugged tube which he carried in his pocket. The lice were fed twice daily by removing the cloth with the vermin clinging to it and placing it upon the back of the head. They can also be kept alive by feeding them twice a day and keeping them in a damp atmosphere in a thermostat at 28°C. Patton and Cragg, on the other hand, fed them only once a day.

Dissection.

The insect is first starved and then rapidly killed in water warmed to 80°C. The insect is then put in 30 per cent alcohol. The louse is placed on a smooth glass slide with the ventral side upwards and is held in position by exerting a little pressure on the thorax with the head of a blunt needle. Then a fine longitudinal incision is made with the help of a sharpened triangular needle on each side of the thorax and the entire abdomen near their lateral margins. After the insect has been pinned on a paraffin tray at both ends, the sternites are removed and the tracheal and other attachments are cut. The alimentary tract can thus be easily exposed.

Prevention.

The prevention of discomfort caused by lice and of diseases carried by them lies in controlling the louse population. The measures are different in cases of head and body lice due to their different modes of life.

Head lice (*P. humanus capitis*). (1) The old time remedy of shaving the

hair constitutes an effective way of dealing with head lice. Cutting the hair, unless it is done close to the scalp, may leave some nits behind. Both cutting and shaving cannot be practised on women. (2) Lauryl thiocyanate and lethane 384 may be used in strengths of 25 per cent and 50 per cent respectively (Busvine and Buxton, 1942). Eggs are not affected. (3) Derris cream made up in 1 per cent strength rotenone may be applied but rotenone is irritating to the skin and does not kill the eggs. (4) Pyrethrum extract (Roy and Ghosh, 1944) is undoubtedly efficacious. The method of treatment is as follows:—Every part of the hair from the root to the tip should be sprayed by means of a De' Vilbiss atomiser No. 15 or 16, with a mixture of pyrethrum extract and kerosene. A concentrated mixture suitably diluted to contain 0.2 mg. percentage pyrethrins has proved very satisfactory. A towel is generally held over the face to protect the eyes. The insecticide is first sprayed on the hair and is then rubbed in the hair in order to ensure uniform distribution of the oil globules that always have a tendency to accumulate on parts directly exposed to the spray. At the end of the treatment the hair may be combed, and lice either dead or in a dying stage may be collected, and preserved for the purpose of studying their population on the head. For those who are to return to clean surroundings, the hair may be washed with soap and water an hour or more after the treatment has been given. (5) A 5 per cent solution of D.D.T. prepared in either crude or more refined kerosene oil may also be used but this will kill lice but not nits. No ill effects are noticed when the scalp is healthy but in presence of seborrheic dermatitis this will cause considerable irritation often resulting in ulceration from scratching. There is constant oozing from the ulcers and sleep is disturbed. When impetigo of the scalp follows pediculosis, one application of pyrethrum and kerosene mixture is recommended. This is followed by the application of 10 per cent pyrethrum ointment two to three times a day. The scalp must be washed with hot water and soap once a day and no oil should be used. Remarkable improvement will generally follow in a short time.

The most efficient way of treating lousiness of the head is by using pyrethrum extract; pyrethrum acts quickly on both the adult louse and the nit and therefore only one application will be necessary.

In connection with the control of body lice it is necessary to remember that eggs are more resistant to cold than the active stages. The longest hatching period recorded is 16 days at 25°C in a dry atmosphere. Dry storage for 2-3 weeks at a temperature of 12-20°C should suffice to dispose of the nits. The nits are killed almost instantaneously in hot or boiling water between 90° and 100°C, and in 5 seconds at 70°C. A dry heat of 160°F will kill lice and nits in 10 minutes.

The two factors which favour the breeding of body lice are (1) the continuous wearing of clothes for days on end especially during the winter, so that the lice are kept at a constant favourable temperature; and (2) the failure to wash the clothes at frequent intervals, so that any stray lice or nits may be killed or removed mechanically.

The different methods by which disinfestation may be effected are the following:

(1) Cold: articles like fur, feather etc., which are likely to be destroyed by heat, either moist or dry, should be left in a refrigerator for 10 days. (2) Warm clothing may be disinfested by storing the garments in a dry atmosphere for one week. (3) Dry heat:—(a) Sunning clothes for disinfestation is not effective. (b) Hot flat iron is especially useful for removing infestation of woollen goods. (4) Moist heat: Both nits and active stages of lice are rapidly killed in hot or boiling water. This is the most economical and useful way of dealing with *corporis*. (5) Immersion in water in order to be effective must be continued for at least five days. (6) A daily bath and frequent change of underclothing is absolutely necessary. (7) The popular belief that anointing the body with oil or fat will afford protection from invasion by lice is not true. (8) The use of ordinary louse powders is a wasteful method of dealing with lice. (9) D.D.T. has been most successfully used against the body louse. Two methods have been employed. The undergarments may be impregnated with a diluted dust or treated with a 5 per cent mixture prepared in kerosene to render them louse proof. The dust is useful for speedy application to large numbers of infested men to check an epidemic of typhus among the civilians. Impregnation of undergarments is most suitable for soldiers. It is the most effective way of keeping a large body of men louse free for an extensive time under difficult conditions. The treatment is imperceptible on the garments.

Genus *Phthirus*.

This genus contains the only species, *Phth. pubis*. This is popularly called crab-lice on account of its general appearance resembling a crab and its powerful legs and claws.

The general features of this insect are the following:

(a) Body is as broad as long; (b) head impacted on the thorax; (c) first pair of legs extremely slender; (d) thumb-like process from the tibia is not developed; (e) the abdominal segments have lateral projections; (f) the second abdominal segment bears three spiracles on each side lying almost in a line.

This species is less frequently encountered on man and is not known to convey any infective disease. It leads to considerable discomfort on account of the irritation of the skin caused by its bite.

The parasites are usually confined to the pubic and perineal regions and even spread to the abdomen, thighs and forearms. They have never been found on the chin or axilla. In young children the louse may be localised on the eye-lashes; this condition is extremely troublesome and leads to blepharitis and conjunctivitis. On rare occasions they are noticed on the temple. The infestation is at times a

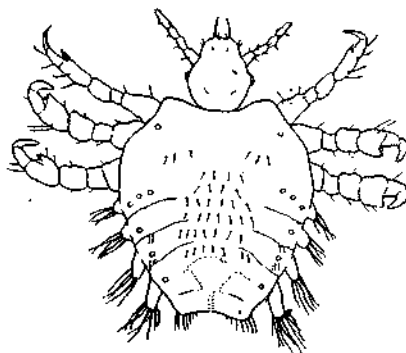


Fig. 132
Phthirus pubis, female.

venereal one but it may be readily acquired from lavatory seats. The louse is generally picked up by direct contact.

Treatment consists in (1) Shaving or cutting the hair ; (2) Spraying with pyrethrum extract ; (3) For eye-lashes, pyrethrum ointment prepared with powdered pyrethrum flowers and vaseline in 8 per cent strength. Pyrethrum does not cause any irritation to the eyes. (Roy, Ghosh & Chopra, 1941).

Animal lice.

In addition to man and monkeys, true sucking lice occur on animals such as buffalo, pigs etc.

Genus *Polyplax*. These are found on rodents. A large number of species have been described. This genus is characterised by the presence of not more than two rows of transverse bristles on the abdominal tergites and sternites.

P. spinulosa Burm. is found on domestic rats, and together with the rat mite it has been suspected of spreading endemic typhus among rats. *P. serrata* occurs on rabbits.

Genus *Hæmatopinus*. They are found on ungulates, e.g., pigs. They are of large size. The vestigial eyes are placed on projecting tubercles ; there is a small triangular platelet between tibia and tarsus.

H. suis L. is found on pigs, *H. tuberculatus* on the buffalo. Genus *Linognathus* is found on dog, sheep and goat and *Pedicinus* on monkey.

Order MALLOPHAGA

(Biting lice)

This is a small order and though the insects belonging to this order are popularly called bird lice, in reality they are more related to Orthoptera.

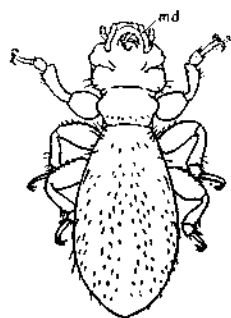


Fig. 133
Mallophaga.
md, mandible.

In general appearance they are flattened, wingless, highly chitinated insects which do not undergo any metamorphosis. The head is unusually large and the mouth parts are represented by a pair of mandibles used for chewing. They feed on epidermis and sometimes on feather. They are generally parasites of birds, and one genus, *Trichodectes*, is found on mammals.

They can be easily distinguished from sucking lice by their large head, mandibles and highly chitinated body.

The eggs of *Dipylidium caninum* passed in the faeces of dogs and cats undergo development to the "cysticeroid" stage in the dog-louse *T. canis* de G. The mechanism of transmission by the dog-louse is the same as by the dog and cat-flea of the genus *Ctenocephalides*.

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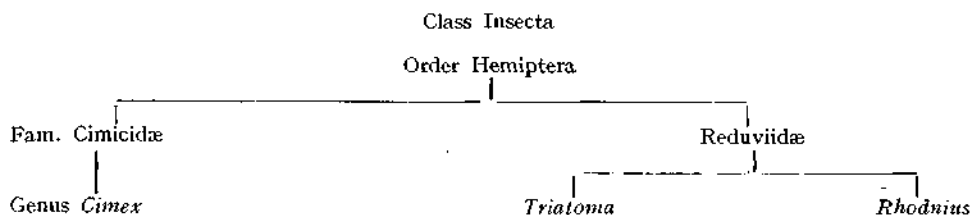
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Order HEMIPTERA (RHYNCHOTA)

(Bugs)



The members of this order are popularly known as bugs which represent a fairly large group of insects of varying sizes. Insects such as scale insects, aphids, cochineal bugs etc., are no doubt primarily important to the agriculturist, but the medical man is interested only in the bedbug and the cone-nosed bug, the former belonging to the family Cimicidae and the latter Reduviidae.

This order can be readily separated from others by the characteristic proboscis. The proboscis or the rostrum is a segmented structure and in repose is kept withdrawn under the head and thorax and is half or fully extended during feeding. Wings may or may not be present; when present they may be two pairs or one pair. Both may be membranous or the basal half of the front pair may be thickened. The mouth is of the piercing and sucking type, and all live on fluid food mostly of vegetable origin. The metamorphosis is incomplete and all are oviparous.

While a large majority of bugs are terrestrial, there are others which are aquatic. Among the latter some belonging to the family Hydrometridæ prey upon mosquito larvæ and other aquatic insects.

Of the many families only two will be mentioned; these are Cimicidae and Reduviidae.

Family Cimicidæ
(True bugs)

These are small, dark brown in colour and are flattened dorsoventrally. All are parasitic and the active stages live on blood alone. Metamorphosis is incomplete. There are usually 1 larval and 4 nymphal stages.

Genus *Cimex*.

Head: antennæ four jointed; eyes simple; rostrum (beak or proboscis) three jointed. The proboscis or the labium encloses the true biting and sucking organs which lie in a deep groove on the dorsal surface. The

mouth organs in bugs are reduced to two pairs of stylets, the anterior pairs are the mandibles and the posterior, the maxillæ. The maxillæ are serrated at their tips and the two maxillæ when they are united have a sharp piercing point. The two mandibles are

grooved on their inner surfaces and when they appose, two tubes are formed, the dorsal one for the passage of blood into the mouth, and through the ventral tube the saliva flows into the wound.

Thorax: consists of the usual three segments. The prothorax is the largest and semilunar. The metathorax bears on its dorsal surface the rudimentary wings in the form of oval pads or elytra one on either side.

The legs bear the usual number of joints except that the tarsi are three-jointed. The last tarsal segment bears the two claws and the empodium.

Abdomen: consists of either 8 or 9 segments, the last two or three being adapted for sexual purposes. In the female following the 6th segment there are four plates, two of which meeting in the middle line form the vagina. The tip of the abdomen is broad in females and narrow in males. The curved penis is accommodated in a notch on the left side of the 8th segment.

The pharynx is narrow. Anteriorly its lumen becomes directly continuous with the food channel formed by the convergence of the stylets of the mouth apparatus. Posteriorly it joins the œsophagus which becomes continuous with the midgut at about the level of the metathorax. The midgut is a long tube and

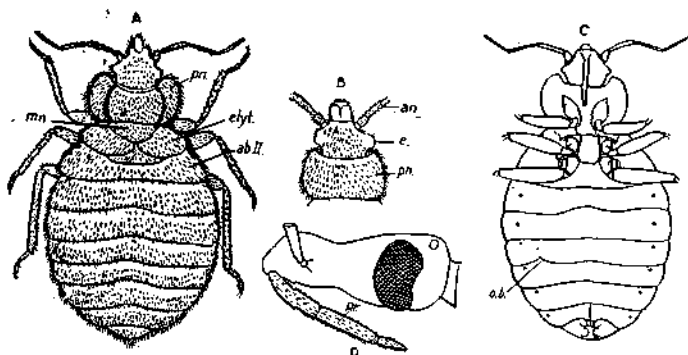


Fig. 134

A. *Cimex lectularius*, female. B. Head of *C. rotundatus*. C. *C. rotundatus*, ventral surface. D. Lateral view of the head of *Triatoma rubrofasciata*.

ab II, second abdominal segment; an, antenna; e, eye; elyt, elytra; mn, mesonotum; o.b, opening into organ of Berlese pn, pronotum.

occupies the largest portion of the alimentary tract. It is divided into an anterior and a posterior part. The hind intestine is globular and consists mainly of the rectum. The junction between the mid and hind gut is marked by four Malpighian tubules.

Stink glands: The offensive smell emitted by a large majority of the members of this order is due to a fluid secreted by a pair of glands, one on each side of the thorax. They open by means of small apertures lying on the metasternum between the second and third pairs of legs.

Respiratory system.

There are two pairs of thoracic and seven pairs of abdominal spiracles. The thoracic spiracles lie between the pro and mesothorax and the posterior pair between the meso and metathorax. The abdominal spiracles are situated on the under surface near to the outer edge.

Reproductive system.

Male: A pair of testes with vasa deferens, vesiculæ seminales, accessory glands with their reservoirs, ductus ejaculatorius and the penis.

Female: Each ovary consists of 7 egg tubes, and the common oviduct terminates at the posterior end of the body in the usual manner between the modified ventral plates so arranged as to permit the expansion of the genital orifice at the moment of oviposition. The spermathecae are pouches that lie on either side of the common oviduct in the region where it is joined by the two lateral oviducts.

"Organ of Berlese": This organ is peculiar to bugs and is found only in females. It consists of a spherical mass, whitish in colour, and lies in the body cavity on the ventral surface of segment 4 and to the right of the middle line. In the right lateral half of the ventral plate of the fourth segment there is a longitudinal slit. This is known as Ribaga's organ. The organ of Berlese is a solid mass of cells with no lumen and no duct. In sections of this organ taken from caught specimens one practically always finds that it contains very numerous spermatozoa. Also striking is the presence of spermatozoa free in the body cavity of the abdomen. Authorities differ greatly on the exact function of these organs. According to Berlese, its discoverer, the spermatozoa are utilised as food material during the sexual life of the female.

The spermatozoa which are extremely long do not enter the posterior genital orifice at the time of copulation; the penis is inserted through the cleft in the right side of the fourth abdominal sternum into the organ of Berlese into which the spermatozoa are injected. They are then transferred to the body cavity and from there to the spermathecae. The genital orifice at the posterior end of the abdomen serves merely as a passage for the exit of the egg at the time of oviposition (Cragg, 1915).

Salivary glands. There are two pairs of salivary glands; one being the gland proper and the other is thought to serve as a reservoir for storing saliva though

from the structure of the tissue it is apparent that both are capable of secreting fluid.

The large gland or the reservoir which is often called the cardiac gland is large and when distended is oval in shape. The duct shortly after emergence from the gland divides into two. One goes towards the mouth and after running parallel with its fellow of the opposite side enters the floor of the mouth at the proximal end of the proboscis. The two ducts open separately.

The other duct which is quite long runs forwards, outwards and then backwards to a small pair of glands, often called oval glands or gland proper, the secretion of which is received into the cardiac glands and after being mixed with the secretion of the latter flows into the mouth.

Cornwall and Patton (1914) found that the small oval glands of *Triatoma rubrofasciata* possess an anticoagulant which prevents the coagulation of blood. The same properties were detected by Puri (1924) in the cardiac glands of *Cimex lectularius*.

Life history.

Metamorphosis is incomplete, the various stages in its life cycle being egg, larva, 4 nymphal stages and adult.

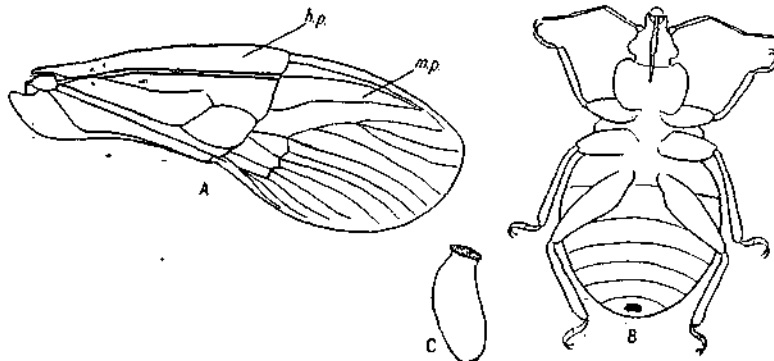


Fig. 135
A. Wing of bug. *h.p.* hardened part. *m.p.* membranous part. B. Nymph of bed-bug.
C. Egg of bed-bug.

Egg: The eggs are white and flask-shaped. The narrower end is closed by an operculum which is perforated with minute holes. The eggs are laid singly in cracks, crevices, furniture, mattress, pillows, hats etc.; in fact they select places well sheltered from light where the bugs also hide in the day time.

In the tropics the minimum time in which the eggs will hatch is 4 to 5 days and in the winter it is extended to 10 days. Blacklock (1912) found eggs of *Cimex lectularius* hatching in 17 days in June, July and August, 14 days at 22°C and 10 days at 25°C.

Eggs are sensitive to cold and if exposed to low temperature, they fail to hatch. The lowest limit of temperature for hatching is 55.4°F. Eggs laid soon

after a meal take longer to hatch than those deposited later. Atmospheric humidity has no effect on hatching (Johnson, 1940).

Ordinarily the eggs can withstand immersion in water at ordinary temperature for at least 4 days. They are not killed when immersed in water for 3 days at between 45° and 50°C or for 48 hours when the water is frozen (Bacot, 1914).

Within an hour or two after emergence from the egg the larva feeds. The bug unlike many other insects feeds intermittently in all stages of its life. The wing pads make their appearance in the first nymphal stage. In all there are four nymphal stages, each stage lasting from 5 to 7 days, at the end of which a skin is cast off.

According to Dunn (1924), *C. rotundatus* differs from *C. lectularius* in that the post-embryonic development is quicker, the adult life is shorter, and the female is more prolific, all of which illustrate the influence of tropical warmth and moisture on insect life.

In the tropics adult bugs can subsist even for three months without food. Thus they may remain alive and active for months in uninhabited houses. But their longevity decreases with rise of temperature. According to Johnson, rabbit's blood appears to be slightly less favourable to survival than human blood. Virgin females live longer than mated ones. The longest survival in houses which had remained empty for a long time was observed by Johnson in England to be between 562 and 572 days.

The weight of blood ingested by *C. lectularius* during one blood meal is 7.6 mg. for the adult female, and 4.9 for the male (Johnson, 1940).

The bed bug is able to crawl easily on rough surfaces such as cloth but its grip on walls is not so firm. Thus it will easily lose its hold when moving on the ceiling. On the other hand, it is believed that the hungry bug is attracted to the host by the smell and that it is common to find them falling from ceilings on to persons sleeping on the floor. All persons are bitten indiscriminately and its bite is extremely irritating to the skin. It will feed readily on any laboratory animal but in nature it shows a marked preference to bite man. It has been a common experience in Calcutta to find bed bugs breeding in association with rats and fleas and also in poultry cages.

Heat is an important factor in stimulating bed bugs to obtain food. According to Rivnay (1932) sebum and cerumen exhibit the greatest attraction.

Bed bugs are disseminated mainly through the agency of personal clothing to which the hungry bug is readily transferred from railway trains, tram cars, furniture, etc. Bedding and furniture are also responsible for the carriage of the bed bug from one place to another.

It is believed that when its source of food supply ceases in any place, it develops migratory habits and escapes by windows and gains entrance into adjoining houses. It is not unusual to find a procession of bugs on the floor at midnight proceeding towards its host. But it is doubtful if they will leave the house and migrate to the neighbour's house.

Bed bugs copulate very frequently during their sexual life. The irregularity as regards oviposition is very striking; usually two or three eggs are laid on the

third or fourth day after the first feed. After one meal of blood nearly three to nine eggs are laid within the first four days and with an unlimited access to food and to the other sex, the number of eggs produced may vary from an average of one per day.

Both sexes of *Cimex lectularius* are fully mature soon after the final moult, and mating may occur before either sex has fed. The eggs do not begin to develop until they are fertilised and only a few are laid after the female has had a meal of blood. Females fertilised by unfed males do not produce so many eggs as those fertilised by fully nourished ones (Cragg, 1923).

Diseases.

A certain number of *Cimex* no doubt succumb when fed on the septicæmic blood of a mouse dying of plague but those which are not killed by the infecting meal are capable of carrying *Bacillus pestis* and re-infecting mice after a period of 48 days' starvation (Bacot, 1915). Cornwall and Menon (1917) have also shown that bugs can under certain conditions transmit plague. However, there is no epidemiological justification for supposing that this takes place to any extent in nature. A large number of other diseases, e.g., relapsing fever, kala-azar etc., have been suspected of being carried by bed bugs, but none has yet been substantiated.

The demonstration of the development of the Leishman-Donovan bodies of K.A. in the intestine of the bed-bug by Patton has now been only of historical interest. It is, however, worth noting that the alimentary tract of the bed bug is in nature absolutely sterile (Patton, La Frenais and Rao, 1921).

Species of *Cimex*.

The two bed-bugs are *C. lectularius* and *C. hemipterus* (*rotundatus*). *C. lectularius* L. is most commonly found in temperate regions while *C. hemipterus* Fab. is peculiar to hot climate. *C. lectularius* is a little larger but otherwise the two are indistinguishable by naked eye. The main distinguishing feature is the character of the prothorax. In *lectularius* it is semilunar in shape, its lateral margins are flat and thin, and the anterior margin has a rounded lobe on each side of the head which projects lateral to the eyes. In *hemipterus* it is narrower and shorter; the margins are rounded and much less expanded laterally; the antero-lateral margin unlike in *lectularius*, just reaches the level of the eye margins.

C. lectularius has spread to America, Australia and South Africa, being carried by man.

C. pipistrelli Jenyns has been found on bats in England, India and S. Africa. It is very hairy and the posterior part of the abdomen is tapering. *C. columbarius* Jen. has been found infesting rat cages in Edinburgh. It infests pigeons. The abdomen is almost circular in outline and the antennæ are much shorter than *C. lectularius*.

C. hirudinis Jen. found in nests of swallows. The body is hairy, the eyes are very small as also are the antennæ.

Family REDUVIIDÆ
(Cone-nosed bugs)

This family of bugs is indeed very large. They are all terrestrial insects, and in size are much larger than bed-bugs. They are found in all parts of the world.

The body is elongate; the head is also elongate. The eyes are placed well forward; the antennæ are longer than in Cimicidæ.

Two genera are important as both are blood suckers.

Genus *Triatoma*.

All are of large size about an inch or more in length. Different species are marked differently on the thorax and abdomen. They are widely distributed in the Oriental Region, Africa and America. At all stages they are blood suckers and man is also attacked.

T. megistus Burm. is a proved carrier of *Trypanosoma cruzi* which produces Chaga's disease in South America. The development of the parasite takes place in the intestine and man becomes infected due to faecal contamination of the wound. According to Neiva the insect is almost domestic in habits and is found only in houses tenanted by man. There are in all five moults before the adult stage is reached. This species is common in Brazil

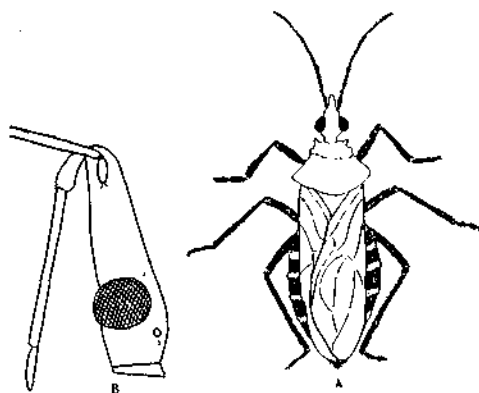


Fig. 136

A. *Triatoma rubrofasciata*.
B. Head of *Rhodnius prolixus*.

and British Guiana..

T. rubrofasciatus Deg. occurs in Asia, Africa and South America. It is often attracted by strong light to dwelling houses and has occasionally been reported as biting man. The female is readily distinguished from the male by the broad and notched tip of the abdomen.

When captured it will often go on laying eggs in batches. The eggs are white when laid but turn pink when mature. The egg will seldom hatch before 30 days. It readily feeds on laboratory animals. There are in all five moults. It was once suspected by Donovan of playing a rôle in the transmission of kala-azar in India.

T. sanguisuga Lec. It is as common in Texas as the bed-bug and bites man freely.

Genus *Rhodnius*.

In this genus the antennæ are inserted well forward near the end of the head; in *Triatoma* the antennæ are inserted further posteriorly. Two species are known to be vectors of *Trypanosoma cruzi*; these are *R. prolixus* Stål in Venezuela, and *R. brumpti* in Brazil. The excreta is infective. They are believed to be more dangerous than *Triatoma* as they defecate immediately after withdrawing their rostrum from the punctures, thus providing more opportunity for infection through them. *Triatoma*, on the other hand, do not defecate until some seconds or minutes

after feeding. It is likely that contamination of the mucous membrane of the mouth, nose or eye may take place with infective excreta.

Dissection of bed-bug.

Allow the bug sufficient time to digest its blood meal before it is dissected. After the legs have been removed the insect is placed in a drop of salt solution on a glass slide. It is firmly held in position with a blunt needle applied on the lateral side of the abdomen. With a sharp-edged needle the lateral margin of the abdomen on both sides except the last two segments is chipped off. The thoracic segments should be treated in a similar manner. It will now be possible to remove the skin either from the dorsal or ventral surface of the body and expose the alimentary canal which is left attached to the head in front and the last abdominal segment posteriorly.

Preservation: All stages of bed-bugs can be preserved in 70-80 per cent spirit. They should never be preserved in a dry state. Reduviid bugs should be mounted on pins.

Poisons against bed-bugs.

Nearly all thin vegetable and mineral oils are toxic to bugs but bugs are capable of walking over a surface soaked with the oil. French chalk is a good insecticide. Pulv. cinchona quickly renders bed-bugs incapable of progression. Naphthalene and benzene are extremely toxic to bugs. Other contact insecticides useful against bugs are, lauryl thiocyanate and n-butyl-carbitol-thiocyanate, and also pyrethrum. Creosote is a good repellent to bugs.

Lauryl thiocyanate and n.-butyl-carbitol-thiocyanate are now being used as contact poisons against the bed-bug. They are poisonous to laboratory animals, but not so when diluted 1: 64 and 1: 40 respectively with kerosene.

Pyrethrum extract when similarly diluted with kerosene and sprayed into cracks will effectively destroy both the adult bug and the egg.

Gaseous insecticides: Powdered insecticides or liquid insecticides are of very limited utility. Gaseous substances offer the best prospect of success. Among them both hydrocyanic acid and SO_2 have good penetrating properties. The places to be fumigated must be air-tight and the room should be sealed up for at least four hours. Both these fumigants are however unsatisfactory from the point of their practical application on an extensive scale.

Methyl bromide has been proved to be lethal to bed-bugs when applied at the rate of 1 lb. per 1,000 cu. ft. at atmospheric pressure. It requires a long exposure from 12 to 24 hours. It is an insidious poison. In France chloropicrin has been extolled as a cimicide.

A mixture of ethylene oxide and carbon dioxide probably will prove a good fumigant for use against bugs.

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Class ARACHNIDA (Ticks, mites, scorpions, spiders etc.)

The class Arachnida includes such creatures as ticks, mites, scorpions, spiders, king-crabs etc. A majority of them are terrestrial and only a small proportion live in water. They possess certain well-defined characteristics. These are:—

(a) The body, as distinct from insects, is not regionally demarcated, the proper segmentation being obliterated. Two distinct parts are seen, the anterior one being the cephalothorax and the posterior the abdomen.

(b) Antennæ are absent. By this character alone this group can be easily distinguished from others.

(c) The mouth appendages consist of: (i) one pair of chelicerae placed in front of the mouth, and (ii) a pair of pedipalps which lie one on either side of the mouth. With the aid of these mouth organs the creature is able to suck fluid food.

(d) In addition to two pairs of mouth appendages, chelicerae and pedipalps, there are four pairs of walking legs.

(e) Metamorphosis is incomplete. Though a large majority is oviparous, there are some which are viviparous.

(f) In this class the larva is characterised by the presence of three pairs of walking legs and the nymph four pairs. The nymph can, however, be easily distinguished from the adult as it is only in the latter stage that the genital aperture makes its appearance.

(g) Sexes are separate.

The respiratory system differs widely among the several orders. Thus scorpions and spiders breathe by means of special organs called lung books; these

open externally on the abdomen. The king crab (*Limulus*) breathes by means of thin lamellæ which are attached to the abdominal legs. Ticks and a majority of mites breathe by means of tracheæ which ramify throughout the body and open externally on the cuticle. Itch-mites and follicle-mites are devoid of tracheæ; they breathe through the integument.

Only a few are parasites, *e.g.*, ticks, mites, and tongue-worms.

The members of the class Arachnida may be classified into four important orders, *e.g.*, Acarina (ticks and mites), Scorpionidea (scorpions), Araneida (spiders) and Pentastomida (tongue worms). The order Acarina may also be subdivided at least into six suborders and these are: (1) Prostigmata (velvet and harvest mites), the spiracles lying on the basis capituli; (2) Mesostigmata (hard ticks), in which the spiracles lie behind the coxæ of the fourth pair of legs; (3) Metastigmata (soft ticks and rat mites), the spiracles lying between the coxæ of the third and fourth pairs of legs; (4) Astigmata (itch mites and also cheese mites), the spiracles being totally absent; (5) Heterostigmata or the soft-bodied mites; and (6) Vermiformia or the worm-like follicle mites.

Superfamily IXODOIDEA.

The superfamily Ixodoidea includes both ticks and mites and is represented in almost every part of the globe. Ticks live strictly parasitic lives and are responsible for animal diseases due to *Spirochæta*, *Babesia*, *Rickettsia* and *Theileria*. A spirochæta of relapsing fever is carried by ticks which also play some part in the spread of typhus-like diseases in various parts of the world. The bite of some ticks is often followed by paralysis and this is particularly noticed in sheep and occasionally in man.

A large proportion of mites, on the other hand, are free living and live on vegetable juice. Only a few are blood suckers and a small number live on the tissue juice of animals including man.

Order ACARINA.

The order Acarina represents both hard and soft ticks. They are all ectoparasites of vertebrate animals and live entirely on blood at all stages of their existence. Many of them act as vectors of numerous protozoal diseases. Some species cause a dangerous paralysis in animals and also in man.

External anatomy.

The body is oval, segmentation being obliterated. At the anterior end lies the false head or capitulum, and posteriorly the abdomen. The capitulum has a rectangular or hexagonal shape which fits into an excavation of the body. From its anterior part projects the proboscis or beak; this is composed of a pair of chelicæ and an unpaired hypostome. The pedipalps lie lateral to the chelicæ and are inserted at the antero-lateral angle of the basis capituli.

The chelicerae are the actual cutting instruments. They are capable of retraction. Each consists of a chitinous bar to the end of which is articulated two stout digits or teeth adapted for cutting the skin. Each chelicera is enclosed in a separate sheath both dorsally and laterally and which is continuous with the basis capituli. On the ventral surface of the chelicerae lies the hypostome which is dart-like and is a median prolongation of the basis capituli; it also is enclosed in a sheath. The distal half of its ventral surface is armed with longitudinal rows of strong recurved teeth giving it the appearance of a file. Proximally it forms the floor of the buccal cavity. The sucking tube is formed by the apposition of the chelicerae above and the hypostome below. Sen (1935) claims to have discovered a separate chitinous tube through which blood passes into the buccal cavity.

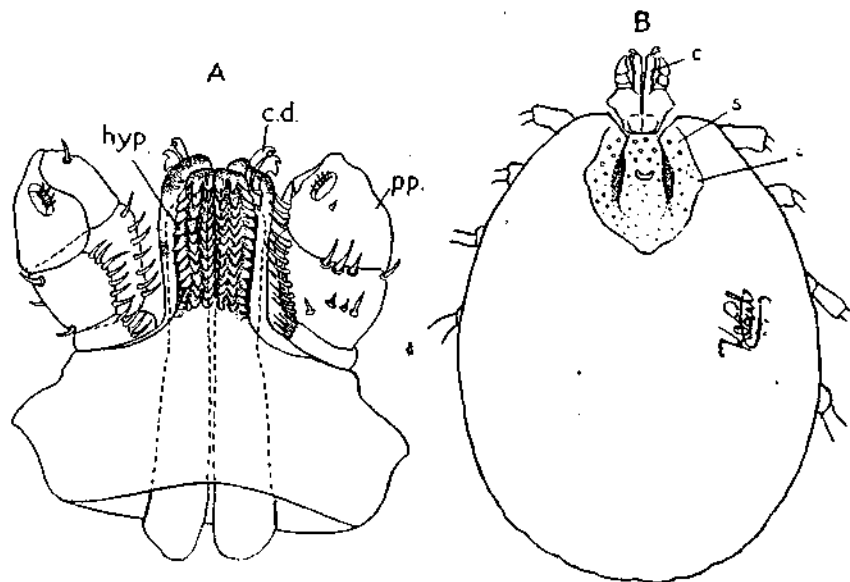


Fig. 137

A. Head of *R. sanguineus*, (ventral aspect). B. *R. sanguineus*, female, (dorsal aspect). c, chelicera; cd, cheliceral digits; e, eye; pp, pedipalp; s, scutum; hyp, hypostome.

Abdomen. The following structures are seen on the dorsal surface: (1) A chitinous shield or scutum, may or may not be present. When present it is larger in males occupying nearly the whole back, and much smaller in females, being confined to the anterior portion only. (2) Eyes may or may not be present; when present they lie on the lateral margin of the scutum. (3) In hard ticks certain grooves are noticeable upon the scutum, especially a pair of marginal grooves. (4) The posterior border of the scutum is festooned conspicuously in the male and in the fasting female.

On the ventral surface are found the following: (1) Genital orifice between the second pair of coxae. (2) Anal orifice lying in the middle line further posteriorly and often surrounded by the anal groove. (3) In the males of certain species adanal plates are found as projecting chitinous ridges on either side of the anus.

(4) Spiracles: their position is different in hard and soft ticks ; in the former they lie posterior to coxa IV and in the latter between coxæ III and IV. (5) 4 pairs of legs. Each leg is composed of (1) coxa (2) trochanter (3) 2-jointed femur (4) tibia and (5) one tarsal segment ending in a pair of claws. On the outer border of the tarsus of the first pair of legs there exists a small pitted structure which is lined with sensory hairs ; it is known as Haller's organ and has been proved to be olfactory in function (Hindle and Merriman, 1912).

Internal anatomy.

The alimentary canal in ticks is different from that found in insects. It is a straight tube which begins at the mouth formed by the apposition of chelicerae and hypostome and terminates in the anus. The pharynx and the oesophagus are small and narrow. The mid-intestine is wide and is provided at its two extremities with cæcal appendages which are considerably distended with blood after feeding.

As ticks can withstand starvation for a long time, these diverticulæ serve as the store-house of food. The hind-intestine is small and very narrow. It ends in the rectal sac. The two malpighian tubules are extremely long in ticks and open into the rectum ; thus the excretory matters are probably carried directly into the rectum.

The mid-intestine of *Argas persicus* contains an anticoagulin but no hæmolysin (Nuttall and Strickland, 1908).

The salivary glands, one on each side, are large, elongated, grape-like bodies. The common salivary duct opens on the floor of the mouth near the base of the hypostome. The secretion of the salivary glands is strongly hæmolytic (Nuttall and Strickland, 1908) and the amount of anticoagulin depends on the time which has elapsed since the tick ingested blood (Cornwall and Patton, 1914).

The coxal glands are peculiar to ticks. They are two in number, one on either side of the body. They are comparatively small and lie above the second pair of coxæ. Each gland consists of the gland proper and a reservoir and the main duct passes between the first and the second coxa and opens externally behind the posterior border of the coxa of the first leg, the opening being concealed by a fold of the coxa. For some reason, which is not known, its secretion is increased while the operation of feeding is in progress ; at that time the ventral surface of the body becomes bathed with the fluid. This is particularly marked in *Ornithodoros*. According to Christophers (1906) the secretion is slightly alkaline and prevents the coagulation of blood. The function of these glands may also have some relation to the excretion of nitrogenous waste products (Robinson and Davidson, 1913). These two tiny glands are capable of secreting a large amount of fluid either during feeding or soon after the feeding has been accomplished.

Reproductive system.

In the female the uterus is a more or less globular organ placed in the middle of the body. The oviducts are convoluted. They open at the two antero-lateral angles of the uterus. The ovary is a large single tube placed across the body. The ovules are grape-like and contain eggs in different stages of maturation. The

vagina is short and opens externally at the genital opening. Into the vagina opens a pair of accessory glands, one on either side.

The Gue's organs, so named by Nuttall after its discoverer, are also peculiar to ticks and are found only in the females in the anterior part of the body, hence they are called cephalic glands. They are concerned in the act of oviposition, and are visible as soon as the anterior end of the dorsal cuticle is reflected forwards. They are primarily concerned with egg-laying when they become considerably enlarged. The egg on being extruded out of the genital canal is rolled on the side of the tick by means of the everted lips of the genital aperture and thus it comes into contact with the secretion of these glands. In this way the egg is carried to the dorsal surface of the tick. This explains why eggs are found over the anterior part of the body of the female tick where the duct opens externally in a common opening which is a broad concentric slit with everted lips and which is situated in the lower part of the camerostomal depression. The camerostome is the cavity enclosing the capitulum.

Life History.

Both sexes are parasitic on animals such as mammals, birds, snakes, and other vertebrates. The metamorphosis is incomplete. The life cycle consists of four stages, (a) egg, (b) larva, (c) nymph and (d) adult. Mating may take place either on the host or on the ground. Eggs are laid in cracks, crevices or in grass adjacent to where the female has dropped off from the host. A very large number of eggs are laid. The oviposition often occupies more than a week. The egg is proportionately large, round and is brown in colour when ripe. Hard ticks deposit all their eggs in a single act of oviposition after which the female dies. The egg seldom hatches before 3 weeks. From the egg emerges the hexapod larva.

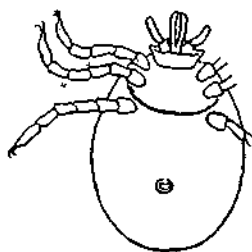


Fig. 138
Larva of *Rhipicephalus*.

The larva does not feed till a week has elapsed after its emergence. It climbs on grass, small shrubs etc. and waits for the chance arrival of its prey. It has ordinarily little tendency to attack any and every animal it finds, but only the proper type of host is selected. On finding one, it immediately attaches itself and begins to feed for some days. When gorged, it drops on the ground and remains quiescent during the time the blood is being digested. At the end of this period moulting takes place. The nymph is provided with 8 legs.

In the same way as the larva does, the nymph seeks a new host, feeds and falls to the ground, where it moults for the last time to be transformed into an adult. After fertilisation and feeding have been completed, the life history begins again.

This is an instance of a three-host tick where larval and nymphal ticks abandon their hosts after feeding. Metamorphosis to the next stage takes place on the ground. Thus they require access to a host on three occasions when they feed in the larval, nymphal and adult stages. *Hemaphysalis leachi* is a 3-host tick. *Rhipicephalus* and *Boophilus* are examples of one host tick for they remain upon a single host throughout their development from larva to replete adult. *R. evertsi*,

which is a 2-host tick, undergoes metamorphosis from larva to nymph upon the host.

At the time of feeding the tick holds its prey firmly with the help of tarsal claws and then makes a rent in the skin by the cheliceral digits. Through this opening the proboscis is inserted, the palps always remaining outside. The teeth pointing backwards and arranged in definite rows on the hypostome help the creature to anchor itself to the host at the time of feeding.

The duration of the life cycle therefore must depend on the ease with which the tick in its different stages can find its suitable prey on which it can thrive. When it fails to find its host, it dies. In the case of the dog tick the cycle from the egg to the adult occupies a little over 2 months. In *Ornithodoros* it occupies about 7 to 8 months.

The position of the respiratory openings and the shape of the body may be used in classifying the order Acarina into different groups or suborders.

SUBORDER.	FAMILY.
1. Prostigmata. (Spiracles placed anteriorly on the basis caputuli.)	Trombididiæ. (Velvet or harvest mites.) The pedipalps with an additional, subterminal finger-like appendix.
2. Mesostigmata. (Spiracles lie behind the 4th coxæ.)	Ixodidæ. (Hard ticks.) Dorsal scutum present and varies in size in the two sexes.
3. Metastigmata. (Spiracles lie between the coxæ of the 3rd and 4th pair of legs.)	(1) Argasidæ. (Soft ticks.) Dorsal scutum absent. (2) Gamasidæ. (Mites). ✓ The chelicerae are strongly exsertile ; pedipalps long and 5-segmented.
4. Astigmata. (Spiracles absent.)	(1) Sarcoptidæ. (Itch-mite of man, birds and domestic animals.) Integument with fine parallel striæ ; legs stumpy, and they terminate in a long stalk ; at the end of some of them is a sucker. (2) Tyroglyphidæ. Integument not striated ; chelicerae chelate and projecting like a rostrum ; pedipalps small. Attack provisions and groceries.
5. Heterostigmata. (Spiracles placed otherwise.)	Tarsonemidæ. The chelicerae are needle-like ; the pedipalps are almost fused with the head.

SUBORDER (*Contd.*)

6. Vermiformia.
(Worm-like ; spiracles absent.)

FAMILY (*Contd.*)

- Demodicidæ (follicle mite.)
Shape of the body worm-like ; 4 pairs of
very stumpy legs ; parasitic in
sebaceous glands of mammals.

In order to avoid confusion, the ticks included in the order Acarina (which includes both hard and soft ticks also mites), may be divided into two families, Ixodidæ and Argasidæ. The former comprises all the hard ticks and the latter the soft ticks. These two families differ not only in structure but also in habits. The difference is shown below.

Difference between the two families, Ixodidæ and Argasidæ.

Fam. IXODIDÆ

(Hard ticks.)

Scutum covers the whole back in the male and only a small part anteriorly in the female.

Capitulum is anterior.

Pedipalps are not capable of movement like a leg.

Spiracles lie behind coxa IV. ✓

Sexual dimorphism is well marked.

Pulvillus is rudimentary. ✓

Only one nymphal stage.

They feed both in the day time and at night.

They are ectoparasites and remain on the host for a long time.

They can not resist starvation for a long time.

Fam. ARGASIDÆ

(Soft ticks.)

Scutum is entirely absent ; hence they are known as soft ticks.

Capitulum lies ventrally and mouth parts are not visible from above.

Pedipalps are leg-like.

Spiracles lie between coxæ III and IV.

Sexual dimorphism is absent.

Pulvillus is well developed.

More than one nymphal stage.

They feed only at night.

They hide like bed-bugs and are parasites only during the time of feeding. They emerge from their hiding places at night.

These ticks are able to live even a year or much longer without any food.

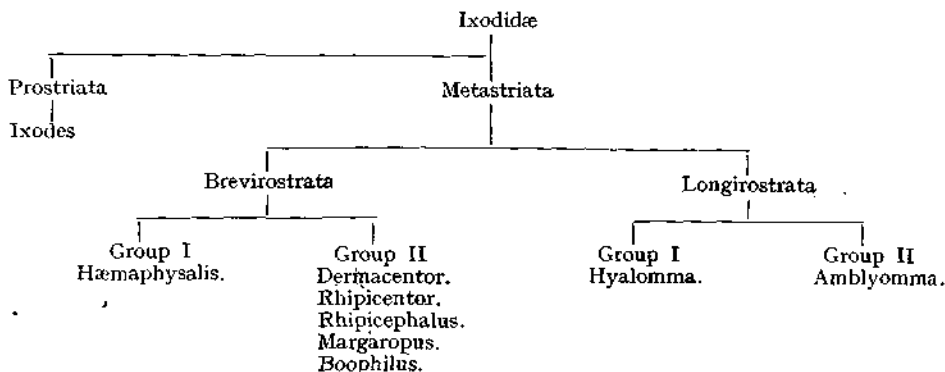
Family Ixodidæ.

The Ixodid or hard ticks are readily recognised by their scutum and also by the capitulum which lies anteriorly. The two sexes can easily be differentiated. The scutum or shield is derived from the integument and becomes highly chitinated. In the male it covers almost the whole back and this prevents the tick from increasing to any appreciable extent in size, whereas in the female it covers only a small part of the back, thus allowing the tick to increase considerably ; the female is therefore much larger.

The capitulum is always terminal in all stages. Eyes, if present, lie at the sides of the scutum.

The nymphs and the adults remain for a week or more upon the host. The females lay several thousands of eggs, feed but once and die after oviposition is completed. They generally do not live long when fed, but when unfed they may remain alive for a long time provided the conditions are favourable. The metamorphosis in 3-host and 2-host ticks take place on the ground.

There are in all 9 genera of Ixodidae and they have been grouped by Nuttall as follows:



Among the 9 genera, two, *Margaropus* and *Rhipicentor*, do not occur in India.

In Prostriata the anal groove surrounds the anus in front, while in Metastrata it lies behind it or is obsolete.

Genus *Ixodes*. It is mainly a three-host tick. The adults are found on a large variety of hosts, e.g., sheep, cattle, dogs, birds etc. They have been recorded from Europe, Japan, North America etc. On rare occasions man may be attacked.

I. ricinus L. is the common sheep tick in Europe. It is suspected of conveying to sheep in Scotland the virus of an encephalomyelitis called "louping ill". It is a three-host tick with a long list of wild and domestic hosts, the important ones being sheep and cattle. It is the common European cattle tick and is responsible for piroplasmosis of cattle caused by *Babesia bovis*. It is suspected of carrying *B. canis* in France. In Europe *I. ricinus* commonly attacks man.

In Australia *I. ricinus* is found on rodents, marsupials and dogs. In *I. holocyclus* Neum., which has been recorded from India, the limbs of the anal groove converge to a point posteriorly.

I. ricinus L., and *I. acutitarsus* Karsch., have been recorded from India attacking sheep, squirrel etc. They are known only as coming from the hilly regions.

Genus *Haemaphysalis*.

Inornate without eyes; festoons present; second article of palp projects laterally; with dorsal spur on the first trochanter; the male shows no ventral plates or shields; spiracles usually ovoid or short comma-shaped.

H. bispinosa Neum., is common on a wide variety of domestic animals in India. *H. leachi* Aud., is the chief carrier of canine piroplasmiasis in South Africa and is specially noticed upon the dog. In Australia it is the principal cattle and horse tick. The palps protrude greatly in this species. The tick's offspring are not capable of conveying the disease until it attains sexual maturity. In other words the infected larvæ are not infective whereas the adults are (Lounsbury, 1904).

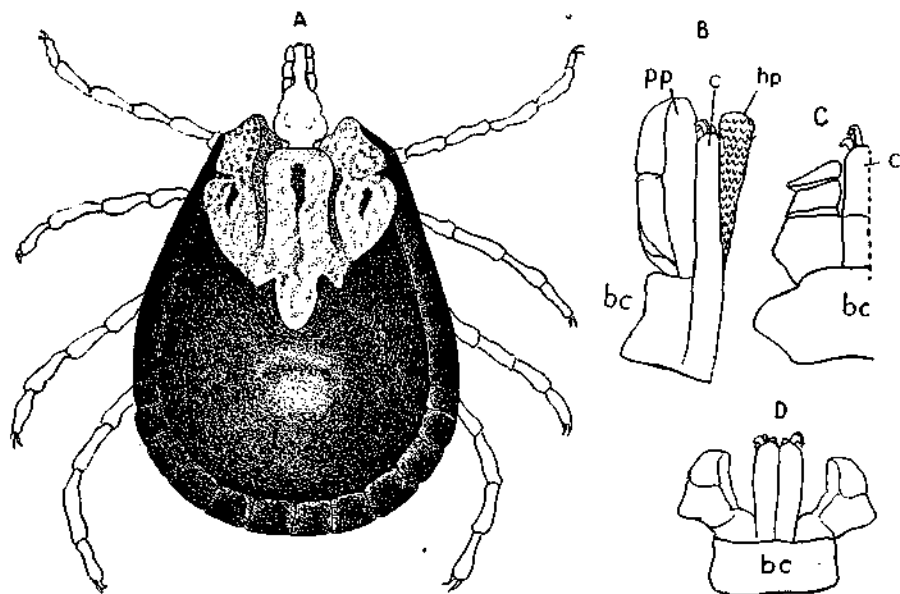


Fig. 139

- A. *Dermacentor andersoni*, female.
 B. Head of *Hyalomma aegyptium*.
 C. Head of *Boophilus australis*.
 D. Head of *Haemaphysalis bispinosa*.
 c, chelicera; bc, basis capitulum; pp, pedipalp; hp, hypostome.

Genus *Dermacentor*.

Usually ornate; with eyes and festoons; basis capituli rectangular dorsally; coxa IV is the largest; coxa I bifid.

The only one of this genus which has attracted considerable attention is *D. venustus* Banks., the wood tick, which commonly attacks man and is the infective agent of the particular type of typhus prevalent in the Rocky Mountains in North America. It is a 3-host tick the adult being found on domestic animals. It causes painless bites to man. The other species which is also responsible for the human cases is *D. variabilis* Say., the common dog-tick in the central and eastern portions of the United States.

Piroplasmiasis in dogs in France is transmitted by *D. reticulatus* Fab. which is found on domestic animals.

In British Columbia, *D. venustus* Banks (*D. andersoni* Stiles) is also responsible for tick-paralysis which affects man, sheep and probably other animals. In Montana, *Bacterium tularensi* has been recovered in *D. venustus* collected under natural

conditions. The bacteria are carried to the progeny (Parker, Spencer and Francis, 1924).

The larvæ and the nymphs of *D. venustus* feed on a wide range of small rodents. The nymphs hibernate and the adult stage is reached the following summer (Cooley, 1923).

Only one species, *D. auratus* Sup., has been recorded from India from wild animals and is of little economic importance.

Genus *Rhipicephalus*.

Inornate; with eyes and festoons; with short palps and basis capituli usually hexagonal dorsally; coxa I bifid; the male possesses a pair of accessory adanal shields; spiracles comma-shaped.

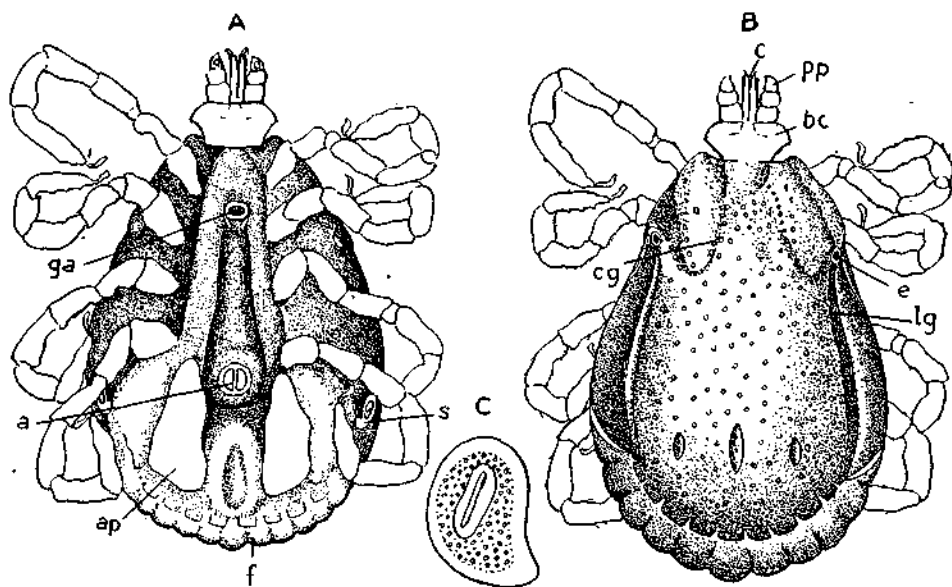


Fig. 140

A & B. Ventral and dorsal aspects of *Rhipicephalus sanguineus*. C. Spiracle.
a, anus; ap, adanal plate; bc, basis capituli; c, chelicera; cg, cervical groove; e, eye;
f, festoon; ga, genital aperture; lg, lateral groove; pp, pedipalp; s, spiracle.

R. sanguineus Latr., a 3-host tick, carries *Babesia canis* in India. Boutenneuse fever of the Mediterranean littoral is also transmitted by this species. Its chief characters are: Scutum with punctations of variable size with three characteristic posterior furrows; adanal shields in male rounded posteriorly. When fully fed the male may show a slight caudal protrusion.

This genus is represented in India by two species, *R. sanguineus* Lat. and *R. hæmaphysaloides* Sup. The former is the most common Indian dog-tick and the latter attacks both wild and domestic animals. The female *sanguineus* is characterised by punctations which are numerous, close-set, irregularly arranged and unequal, larger ones being absent in the posterior portion of the median field,

whereas in the female *hæmaphysaloides*, the punctations are free, sparsely scattered, and strongly unequal; the larger ones are arranged in longitudinal rows and found in the posterior portion of the median field, finer ones being hardly visible.

This tick, as has been proved by Christophers (1907), is the transmitting agent of canine piroplasmiasis in India, the disease being heritable in the tick, latent in the larval stage, and maturing in the nymphal and adult issue of the females that acquired the infection.

R. appendiculatus Neum. is responsible for the Rhodesian East Coast fever caused by *Theileria parva*. The parasites are not transmitted hereditarily in the tick but only from one stage to another. This species is common in South Africa. It resembles *R. sanguineus*; the tail-like projection at the posterior end of the body is very distinct; coxa I is visible dorsally; punctations very numerous and evenly distributed, a few large ones being placed anterior to the lateral groove.

R. evertsi Neum., a 2-host tick, is common in South Africa. It is readily recognised by its eyes which are rounded and convex and lie in distinct orbits; the legs are bright orange red. It carries equine piroplasmiasis in South Africa. It is not known whether it carries *B. caballi* or *B. (Nuttallia) equi*.

R. simus Koch., a 3-host tick, is also common in Africa and is responsible for the carriage of *T. parva* to cattle. It is a stage to stage infection as in *R. appendiculatus* Neum., which is the usual carrier in Africa. Ovine piroplasmiasis is conveyed by *R. bursa* in Persia and Rumania.

Genus *Boophilus*.

Boophilus is essentially a cattle tick though found on other animals also. It is widely distributed and carries bovine piroplasmiasis to cattle.

In India this genus is represented by 2 species, *australis* Fuller and *annulatus* Say., the former being more common than the latter and represents the Indian form of the Texas fever tick.

The causative organism of Texas fever is *Babesia bovis* or *B. bigemina* and is transmitted by *B. annulatus* and *B. decoloratus* Koch. Cattle are infected with *Babesia* by the offspring of an infected tick. The parent tick is not therefore a direct carrier, but as the infection passes through the eggs, its chances of reaching a susceptible animal are enormously multiplied. In North America the chief carriers of Texas cattle fever are *Boophilus australis*, and *Margaropus (Boophilus) annulatus*, whereas in South Africa the principal carrier is the blue tick, *Boophilus decoloratus*. In parts of Europe an identical type of the disease is transmitted by *Ixodes ricinus*.

In regard to *B. decoloratus* acting as an important transmitter of *B. bigemina* in Africa, it has been observed that the tick retains the infection even when it has been reared for several generations on hosts that are not susceptible. Hereditary transmission has also been discovered in *Rhipicephalus bursa* C & F.

B. decoloratus Koch., a one-host tick, occurs throughout tropical and southern Africa on cattle. This tick does not live long unfed and it has been most successfully eradicated by dipping processes and changing the pasturage. It is commonly called the blue tick on account of its characteristic appearance.



Genus *Hyalomma*.

Pedipalps long; eyes distinct; festoons present; stigmata comma-shaped; legs banded; the male with adanal shields and paired chitinated points borne on posterior abdominal protrusion.

The commonest species is *H. aegyptium* L. which is widely distributed in Africa, also in this country and is found on cattle and horses. The female can be easily recognised by its dark brown scutum with prominent eyes placed well inside its margin. Coxa I in both sexes is deeply cleft. The adanal plates of the male are much longer than broad and are provided with chitinated points posterior to them. The legs are marked white at the joints. The female when replete attains a very large size.

H. aegyptium is more or less cosmopolitan. It is suspected of being the arthropod host of the piroplasma-like parasites causing biliary fever in horse. It is the dog tick in Australia. It is a three-host tick. In India it has been experimentally proved by Ray (1945) to be the vector of theileriasis (due to *T. annulata* infection) amongst calves.

The number of eggs laid by a female *aegyptium* is from 6,000 to 8,000 during about 20 days, the maximum number in twenty four hours being 800. The female dies before the larvæ hatch. The incubation period of eggs is from 23 to 26 days. The larvæ feed after a resting period of nearly 20 days; they are found on the ears and other soft parts of the horse, calf, camel, buffalo etc. The larva feeds for 3 to 5 days and then drops on the ground; the nymphs emerge after 5-7 days and are ready to attach themselves to a host after a rest period of 5 or 6 days. They feed for 5 to 7 days and leave the host, the adults emerging after 9 to 15 days rest. After another 7-8 days they are ready to reattach themselves. (Sharif, 1924).

Genus *Amblyomma*.

Scutum ornate with eyes; pedipalps long; festoons present; stigmata broadly triangular with rounded angles; males without any ventral armature but the scutum is more beautifully ornamented than in the female.

They are widely distributed and are found almost on any type of vertebrate, their host relationship being very loose.

This genus is represented by 7 species and is found mostly on reptiles, tigers etc.

A. americanum Lin., the lone star tick, so called because of the prominent star-shaped marking on the back in the female, is widely distributed in both North and South America occurring on a wide variety of animals particularly on the head and ears of wild deer and grouse. Next to *Boophilus annulatus*, it is the most important animal tick in the United States.

This species was found to be the vector of a typhus-like fever discovered in South Texas similar to Colorado tick fever, and to which the name of Bullis fever was given from camp Bullis in Texas where the disease was first observed by Woodland etc. (1943).

A. cajennense Fab. is a notorious pest to travellers in the bush during the

dry season in Panama ; they are very abundant in Texas and attack man and animals indiscriminately. It also occurs in Central and Tropical America. Horses and mules are commonly attacked. Eyes flat ; the first coxa has 2 short spines ; scutum of male has about 9 elongate yellowish spots. In *A. hebraeum* the festoons are whitish, the two spots at the sides are yellowish, the greater part of the light areas are pale violet or pinkish. In South Africa it carries "Heart water" caused by *Rickettsia ruminantium*, a frequently fatal disease in sheep, goats and cattle. The disease and the tick are confined to Africa. *A. variegatum* Fab., another South African tick, is also responsible for the carriage of the same disease.

Genus *Rhipicentor*. Inornate with eyes and festoons with short palps ; basis capituli hexagonal and having prominent lateral angles ; coxa I bifid ; the male resembles *Dermacentor* ventrally and *Rhipicephalus* dorsally.

Genus *Margaropus*. (*Boophilus* of American authors.)

Inornate with eyes, but without festoons ; with short palps and capitulum intermediate between that of *Rhipicephalus* and *Boophilus* ; highly chitinised ; the articulations of the legs greatly swollen especially of leg 4 ; tarsi with a long spur extending far beyond the claws.

M. annulatus, found on cattle, is of considerable economic importance in North America.

Genus *Aponomma*. These are *Amblyomma* but without eyes. They attack reptiles.

Ticks Biting Man.

The following ticks have been reported by Strickland and Roy (1939) as biting man in India.

Amblyomma sp. (nymph).

Dermacentor sp. (nymph).

Hæm. bispinosa Neum.

Hæm. aculeata Lav.

Hæmaphysalis sp. (nymph).

Hyalomma aegyptium L.

Hæmaphysalis montgomeryi Nuttall.

Ixodes acutitarsus Karsch.

Rhip. sanguineus Lat.

Rhip. hæmaphysaloides Sup.

In addition to the above, Kirwan (1935) found *D. auratus* Sup. on upper eyelid of man.

Ticks and Domesticated Animals.

The following is a list of ticks which commonly infest domesticated animals in India.

Boophilus australis Fuller.

Hæmaphysalis bispinosa Neum.

Hyalomma aegyptium L.

Rhip. sanguineus Lat.

Rhip. hæmaphysaloides Sup.

HARD TICKS AND HUMAN DISEASES.

Tick paralysis. A paralysis of man and animals associated with the presence of ticks on the body has long been known to occur in Australia, British Columbia and in other countries. The onset is insidious and though the course of the disease is slow, it may end fatally unless the tick is removed.

In Canada, Hadwen and Nuttall (1913) experimentally established the connection between tick and tick paralysis in sheep with *Dermacentor venustus*. This tick is also considered to be responsible for tick paralysis in Montana. Later, Dodd (1921) experimentally produced tick paralysis in dog with *Ixodes holocyclus* Neum, and Ferguson (1924) recorded a fatal case of paralysis in a child due to the same tick. Other ticks; e.g., *Ixodes pilosus* Koch and *I. ricinus* Lin. are suspected of causing tick paralysis in South Africa and Crete respectively.

Even a single tick is able to cause a fatal paralysis. It has been suggested that the causal factor in the production of the disease is a toxin derived from the salivary secretion (Ross, 1926), the poison in the eggs being identical with that secreted by their salivary glands. The toxic properties contained in the eggs and ovaries of ticks when injected in animals produce symptoms of paralysis together with degenerative changes in the spinal cord (Regendanz and Reichenow, 1932; Hoeppli, R and Feng, L.C, 1933). It has also been shown that the eggs of certain South African ticks are toxic to guinea pigs (De Meillon, 1942).

Tularæmia in man. Tularæmia, which has recently been described in North American rodents, is a plague-like fatal disease and its causal organism is *Bacterium tularensi*. It has been found that human beings also are subject to this disease. It is believed that the transmission to man may be effected mechanically by any blood-sucking Diptera, ticks, mites and fleas. Experimentally the rat-louse *Polyplox* is also able to carry the disease to healthy rats.

This disease has been reported from almost every state in the U.S.A. with the exception of two. In Montana and surrounding States infection has been traced to *Dermacentor venustus*, Banks (*andersoni* Stiles), in the southern states to *D. variabilis* Say., and in Utah and the neighbouring states to *Chrysops discalis* Will.

Typhus.

(a) Rocky Mountain spotted fever. This disease which is confined to certain parts of the United States, such as Idaho, Montana, Wyoming, Utah and neighbouring provinces, is a typhus-like fever and is carried by *Dermacentor venustus* Banks (*andersoni*) in the western parts, and *D. variabilis* Say. in the eastern and central portions of that country. A large number of other species have been suspected and these have more or less only local importance. The infection may be transmitted by the larva, nymph, and both adult female and male ticks. Only the adult tick attacks man but the disease is transmitted to animals such as the mountain goat and sheep and other animals, by larvæ and nymphs. The infective agent is also transmitted by heredity to the ticks through their larvæ. Da Rocha Lima (1916) first described the causative agent of Rocky Mountain fever constantly found in the tissues of human cases as well as in the tissues and eggs of infected ticks. The organism has been designated as *Dermacentroxenus rickettsi* (Wolbach, 1919)

but more commonly known as *Rickettsia rickettsi*, which lives intracellularly in the tick. These organisms are passed with the faeces of infected ticks.

(b) Sao Paulo fever in Brazil, which is really Brazil spotted fever, is transmitted by *Amblyomma cajennense*.

(c) Tick typhus in India is supposed to be carried by a tick, and *R. sanguineus* has been a suspect (Megaw, 1921).

(d) "Bullis fever" in South Texas is carried by *Amblyomma americanum*.

(e) Q. fever in Australia has recently been observed in abattoir workers and dairy farmers in Victoria and is carried by *Ixodes holocyclus*, which is also responsible for tick paralysis in man and domestic animals, its principal host in Australia being the bandicoot *Isodon torosus* and *Perameles nasuta*. *I. torosus* is a proved reservoir of Q. fever. Natural infection has been found in nymphs and adults of *I. holocyclus*. The infection in ticks passes from larvæ to nymphs, from nymphs to adults but not from females to their progeny. Infection in bandicoots can take place by feeding on infected ticks. The rickettsia concerned is *R. burneti*, found in the lumen of the gut and the cytoplasm of the epithelial cells.

The two commonest ticks on bandicoots *I. torosus* are *Hæmaphysalis humerosa* Warb. & Nutt., and *I. holocyclus* Neum. *H. bispinosa* Neum has also been artificially infected and it is possible that it may also carry the disease to cattle from bush animals. Excreta of ticks is infective. *H. humerosa* is a proved vector which carries the disease from cattle to cattle.

Rickettsiæ in Man.

The table given in page 305 represents the pathogenic rickettsiæ found in man. Their distribution and the different vectors have also been included. (Vide Lancet, 1942, Jan. 21, p. 149).

Rickettsiæ in Arthropods.

In addition to pathogenic rickettsiæ, non-pathogenic rickettsiæ have been described in a large number of insects, such as *Mallophaga*, *Anoplura*, *Cimex lectularius*, *Culex pipiens*, *Ctenocephalides felis*, *Ctenopsylla musculi* etc. Cowdry (1923) has examined 111 species of arthropods including spiders, ticks, mites etc. and 13 orders of insects, and claims to have found intracellular, Gram-negative, bacterium-like rickettsia in 19 of these species.

According to Hertig and Wolbach (1924) the generic term "Rickettsia" should be restricted to intracellular pleomorphic forms which are admittedly pathogenic to man. The author has classified rickettsiæ as follows though it has been admitted that such classification is not very sound.

(1) Intracellular forms exhibiting coccoid, rod and filamentous phases, e.g., *R. prowazeki*, *Dermacentroxenus rickettsi* and the rickettsiæ of the bed bug and *Culex pipiens*.

(2) Intracellular, uniform coccoid and diplococcoid forms, e.g., of *Culicoides* and the book-louse *Dorypteryx*.

(3) Forms in which the dominant phase is extracellular in the lumen of the host's gut, e.g., *R. pediculi*, *R. melophagi*, and the rickettsiæ of the book-louse (*Psocus*) and of various *Mallophaga*.

Rickettsiae in MAN (Vide p. 304).

Disease	Rickettsia	Geographical distribution	Insect vectors	Possible vertebrate reservoirs
Exanthematic typhus	<i>R. prowazeki</i>	Europe, Abyssinia, N. Africa, Belgian Congo, Asia Minor, Persia, North China, Mexico.	Louse <i>Pediculus humanus</i>	Man.
Endemic or murine typhus	<i>R. prowazeki</i> var. <i>mooseri</i> (<i>R. muricola</i>)	World wide.	Rat flea <i>Xenopsylla cheopis</i>	Rat (squirrel, shrew).
Tsutsugamushi disease	<i>R. orientalis</i> (<i>R. tsutsugamushi</i>)	Japan, Formosa, Malaya, Java, Sumatra, New Guinea.	Larva of <i>Trombicula akamushi</i> (Japan) <i>T. deliensis</i> (Malaya). <i>T. deliensis</i> (India). <i>T. minor</i> (New Guinea).	Bandicoot.
Trench fever	<i>R. quintana</i> (<i>R. volhynica</i> and probably <i>R. weigli</i>)	North Africa.	<i>P. humanus</i>	Man.
Rocky Mountain spotted fever (Eastern and Western forms)	<i>Dermacentor rickettsi</i>	U.S.A.	<i>Dermacentor andersoni</i> , <i>D. variabilis</i>	Goats, hares, and other small rodents.
Fievere bouton-neuse	<i>D. rickettsi</i> var. <i>canori</i>	Mediterranean zone.	Dog tick <i>Rhipicephalus sanguineus</i>	Dog.
South African tick-typhus	<i>D. rickettsi</i> var. <i>peiperi</i>	South Africa.*	Tick <i>Hæmaphysalis leachi</i>	Dog.
Sao Paulo rural typhus (Brazil spotted fever)	<i>D. rickettsi</i> var. <i>brasiliensis</i>	South Brazil.	Tick <i>Amblyomma cajennense</i>	Opossum.
"Q" fever	<i>R. burneti</i> (<i>R. diaphorica</i>)	Australia, U.S.A.	Ticks <i>Hæmaphysalis humerosa</i> , <i>Dermacentor andersoni</i> , <i>D. occidentalis</i> , <i>Amblyomma americanum</i> , <i>Rhipicephalus sanguineus</i>	Bandicoot.

*Tick-borne typhus also occurs in Abyssinia and in Kenya where the dog tick *Rhipicephalus sanguineus* acts as a vector.

The relationship between the different forms of typhus fever has been shown by Megaw (1942) as follows.

Epidemic typhus fever (a human disease). Transmitted from man to man by human lice. Non-epidemic or zootic typhus (primarily animal diseases). Transmitted secondarily to man by fleas, ticks or mites.

Names	Louse typhus	Flea typhus (Murine, epidemic typhus, shop or urban tropical typhus)	Tick typhus, Rocky Mountain spotted fever, tick-bite fever, etc.	Mite typhus.
Virus	<i>R. prowazeki</i>	<i>Rickettsia mooseri</i>	<i>Rickettsia rickettsi</i> (<i>Demacentroxenus rickettsi</i>)	<i>R. tsutsugamushi</i>
Vectors	Head and body lice	Rat fleas	Various ticks	Larval mites
Reservoirs of infection	Man	Rats & mice	Various rodents, and ruminants; dogs also suspected.	Rats
Agglutination against Proteus	O X 19 + + to + + + O X 2 + + to + + + O X K - to +	+ + to + + + + + to + + + - to +	- to + + + - to + + - to + +	- to + - to + + + to + + + +
Distribution	Cosmopolitan	World-wide, common in the tropics.	Many localities in America, Europe, Africa and Asia.	Far eastern tropical and subtropical countries.

N.B.—The Indian variety of mite-borne typhus has not yet been definitely proved to be carried by *T. deliensis*; it has, however, been strongly suspected.

HARD TICKS AND ANIMAL DISEASES:

In addition to human diseases, a large number of animal diseases are also carried by ticks (vide list below). The transmission of such diseases by tick is intimately associated with its mode of life. Where the tick has only one host, the disease can be transmitted to that host only, but if the tick requires two or three hosts, it is possible for the same tick to transmit the disease to different animals.

The principal danger of tick-borne diseases is on account of the capacity of ticks to transmit the infection to their progeny. This is the case with piroplasmosis, anaplasmosis and spirillosis. Sometimes the infection acquired from the parent does not reappear except in the adult of the next generation. Thus *H. leachi*, though capable of hereditary infection, is not infective till the adult stage is reached.

The following is a list of diseases of domestic animals and carried by hard ticks.

Disease	Tick	Distribution
Canine piroplasmosis (<i>B. canis</i>)	<i>Rhip. sanguineus</i> <i>Ixodes ricinus</i> <i>Hæmaphysalis leachi</i> <i>Dermacentor reticulatus</i>	Asia. Europe. South Africa. Europe (France).
Bovine piroplasmosis (<i>T. parva</i>)	<i>R. appendiculatus</i> <i>R. evertsi</i> <i>R. simus</i> <i>Boophilus decoloratus</i> <i>B. australis</i> <i>R. bursa</i>	Africa. " " North America. India. Africa.
Red water in cattle due to (<i>B. bovis</i>)	<i>B. annulatus</i> <i>B. australis</i> <i>B. decoloratus</i>	N. America and Australia.
(<i>B. bigeminum</i>)	<i>B. decoloratus</i> , <i>R. bursa</i> <i>I. ricinus</i>	Africa. Europe.
Ovine piroplasmosis (<i>B. ovis</i>)	<i>R. bursa</i>	Rumania, Turkey.
Equine piroplasmosis (<i>B. caballi</i>)	<i>D. reticulatus</i> <i>R. evertsi</i>	Russia. Africa.
Nuttallia (<i>B. (Nuttallia) equi</i>)	<i>R. evertsi</i>	India, Africa and Brazil.
"Heart water" of cattle (<i>Rickettsia ruminantium</i>)	<i>Amblyomma hebraeum</i> <i>A. variegatus</i>	S. Africa. "
Encephalitis in sheep (Louping ill)	<i>I. ricinus</i>	Europe.
Anaplasmosis of cattle.	<i>Amblyomma marginale</i> <i>B. decoloratus</i> <i>R. bursa</i> and other ticks	America. Africa. "

While bacterial and virus diseases are mostly carried by flies, ticks are mainly responsible for the carriage of protozoal diseases of domestic animals (except *Phle-*

botomus, which carry *Leishmania* infection to dogs). The different protozoa affecting animals in India and carried by ticks are the following.

Genus *Babesia*. The genus *Babesia* is transmitted through the agency of ticks alone in which cyclical development of the parasite takes place. The life cycle of *B. canis* in *Rhipicephalus sanguineus* has been worked out by Christophers (1907), and Shortt (1936). It has been shown by Shortt that the infection is transmitted hereditarily by tick.

Genus *Theileria*. *T. parva* is the cause of East Coast Fever of cattle in Africa and elsewhere. The species pathogenic to cattle in this country is *T. annulata* which is different from *T. parva*. Ray (1945) points out that *H. ægyptium* is the intermediate host and the infection in ticks is transmitted hereditarily to the succeeding generations. He further states that the infection in the vertebrate host can be produced by the bites of adults only and not by the earlier stages such as the larva or the nymph.

Genus *Pattonella*. This genus has recently been created by Ray and includes only one species, *P. gibsoni* (*Piroplasma gibsoni* Patton), which was first reported from dogs and jackals from Madras. Swaminath and Shortt (1937) have shown that the infection is transmitted through the bite of the ticks *Hæmaphysalis bispinosa* in addition to *R. sanguineus* (Sen, 1933).

Genus *Anaplasma*. Only *A. marginale* has so far been reported from India. It produces "gall sickness". It is probably carried by any blood-sucking arthropod among which ticks must be considered as the most important.

In connection with anaplasmosis caused by *Anaplasma marginale*, *B. decoloratus* is the most important vector in Africa and though the disease can be transmitted by a large number of ticks of other genera such as *Amblyomma*, *Hyalomma*, *Dermacentor*, and *Rhipicephalus*, hereditary transmission has, however, only been proved to occur in *R. simus* and in some varieties of *B. annulatus*. Rees (1934) found stage to stage infection in *R. sanguineus* and hereditary transmission in *B. annulatus*.

The following key prepared by Warburton has reference only to the ticks commonly found on domestic animals in this country.

FEMALES.

- | | | | |
|---|-----|-----|---------------------------------|
| 1. The whole rostrum twice as long as broad | ... | ... | <i>Hyalomma ægyptium</i> . |
| The whole rostrum quite or nearly as broad as long | ... | ... | 2. |
| 2. Rostral base rectangular and narrower than the width across the palps. | | | |
| No eyes. | ... | ... | <i>Hæmaphysalis bispinosa</i> . |
| Rostral base hexagonal, pointed laterally. Eyes. | ... | ... | 3. |
| 3. Peritreme nearly circular, scutum elongate and smooth | ... | ... | <i>Boophilus australis</i> . |
| Peritreme sub-triangular, scutum as broad as long and punctate | ... | ... | <i>Rhipicephalus</i> . |

MALES.

- | | |
|---|--|
| 1. The whole rostrum twice as long as broad | <i>Hyalomma ægyptium</i> . |
| The whole rostrum quite or nearly as broad as long | 2. |
| 2. Rostral base rectangular and narrower than the width across the palps. | |
| No eyes or anal plates | <i>Hæmaphysalis bispinosa</i> . |
| Rostral base hexagonal, pointed laterally | 3. |
| 3. All four anal plates well developed and linear, abdomen ending in a sharp point | <i>Boophilus australis</i> . |
| External anal plates mere chitinous points | 4. |
| 4. Internal anal plates rounded | <i>Rhipicephalus sanguineus</i> . |
| Internal anal plates hooked towards one another | <i>Rhipicephalus hæmaphysaloides</i> . |

Family ARGASIDÆ ✓

(Soft ticks)

All members of this family, like those of Ixodidæ, are parasitic and live on blood alone. The nymphs and adults are rapid and frequent feeders. Like bed bugs, they hide in cracks and crevices in the day time and emerge at night to feed on the host. The females lay a moderate number of eggs in batches after each meal of blood. These ticks can fast for 3 to 4 years.

The Argasidæ comprise two genera, *Argas* and *Ornithodoros*, and their difference is shown below.

Difference between the genera of Argasidæ.

Genus ARGAS

1. Body is oval.
2. Margin of the body is flat.
3. Chitinous plates arranged in radial lines are found on both surfaces.
4. Legs are smooth.
5. Eyes are absent.

Genus ORNITHODORUS

1. Body is much rounder.
2. Margin is round ; a lateral notch between cephalothorax and abdomen present.
3. Chitinous plates are absent ; integument mammilose.
4. Distal segments of legs provided with tubercles on the anterior border.
5. Eyes may or may not be present.

Genus ARGAS.

The body is oval and covered with discs of all sizes on both surfaces.

Argas persicus Oken: is cosmopolitan in warm countries and is found especially in poultry houses but seldom on fowls themselves. It transmits the

spirochaete, *Spirochaeta anserina*, of fowls and ducks which causes heavy mortality among them. It is one of the most troublesome pests which affect poultry, killing enormous numbers of birds from the loss of blood. It attacks poultry at night and is often found on the walls and roofs of poultry sheds in the day time. It also hides in cracks and crevices even in the timber itself.

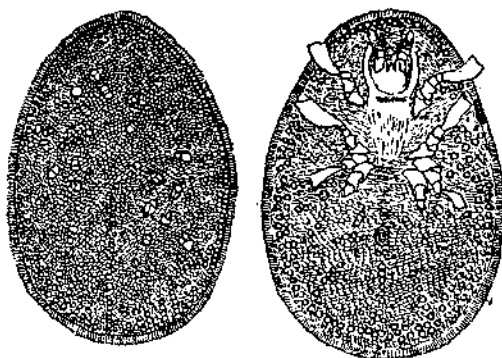


Fig. 141
Argas persicus, dorsal and ventral aspects.

The adult tick is brown in colour and the body is elliptical; its lateral margin is distinctly flat and is formed by rectangular cells.

The larval forms are very minute, often colourless. These larval ticks climb the perches and walls of the poultry house and so reach the roosting fowls, to which they remain firmly attached until gorged with blood. Both young and adult stages of these ticks can remain alive for long periods without food. The adult feeds once a

month and appears to require that period for the digestion of the blood. The adult lays eggs in small batches of 20 to 100. From egg stage to egg stage it may take as long as 10 months. Adult ticks can travel great distances and in this way they migrate to new fowl houses. The species infests native dwellings in Persia and their bites are painful.

Disease: Spirochaetosis of fowls caused by *Spirochaeta anserina* is carried by *A. persicus*. Infection is normally transmitted by the saliva and bite of the tick but occasionally the secretion of the coxal glands is also infective. Infection of glands may take place as early as the sixth day and the bite of the tick is infective on the seventh day. Eggs also become infected in the ovary and hereditary transmission of the spirochaete is known to occur.

Fowls become infected when bitten by infected individuals of *A. persicus* or when infected ticks or eggs laid by them are ingested (Knowles, Das Gupta and Basu, 1932).

Aegyptianella pullorum, piroplasma-like parasites inhabiting the red blood cells of fowls, is transmitted by *A. persicus*.

Pasteurella avicida, the causal organism of chicken cholera or fowl plague is carried by *Argas persicus*. The excreta is infective and the excreta of infected birds are a cause of contagion to healthy birds. Basu (1930) refers to the possibility of the infection being acquired by ingestion though there is marked loss of virulence of *P. avicida* in the gut of the tick.

Argas reflexus Fabr.: It is the pigeon tick of Europe, North Africa and North and South America and kills by massive infestation. It closely resembles *A. persicus* in appearance and habits but differs from the latter in having the anterior end of the body more sharply narrowed, and the margin being finely striate.

It is only when man lives in close association with pigeons or when the pigeon house has been abandoned, that this tick is attracted to a new host, and thus man gets bitten. Besides pigeons, they are known to attack ducks, geese, sparrows etc.

In addition to *A. persicus*, *I. vespertilionis* Koch has been reported from the Punjab as attacking birds.

Genus ORNITHODORUS.

O. savignyi Aud.: In Northern Africa this tick is found buried in the soil in or around the huts on the outskirts of coastal towns especially in the more squalid insanitary areas. The ticks are common in the dusty soil and burrow to a depth of $\frac{1}{2}$ to 1 inch lying dormant for months until they are disturbed by man or beast. The best and the cheapest method of destroying them is to cover the infested areas especially around wells with dry grass and brush wood after harrowing the surface and then setting fire to the grass. It is chiefly parasitic on camels in Africa, but will occasionally bite man, and may also carry the spirochæte of relapsing fever.

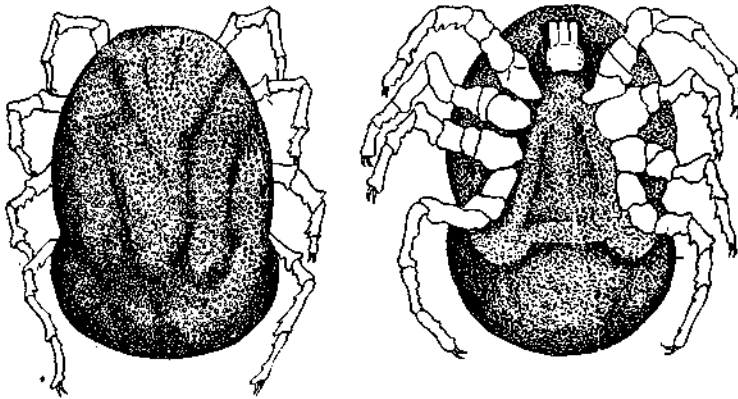


Fig. 142
Ornithodoros savignyi, dorsal and ventral aspects.

In India it is found in the southern parts of the Peninsula. It generally attacks cattle and other domestic animals. It occasionally bites man; when it does, the younger stages are generally involved.

There are two pairs of eyes on each side and in this respect it differs from *O. moubata* which is eyeless. In other respects the two are indistinguishable.

O. moubata, Murray: Like *O. savignyi* the body is oval, rounded anteriorly and is constricted in the middle; the integument has a granulated appearance; eyes absent; distal segments of legs armed with tubercular protuberances on the outer border.

This species is common in Africa and infests huts and rest houses. They hide in the day time in cracks in the mud floors and in crevices in the walls and emerge at night to feed. It is said to be carried long distances in beds and other belongings by travellers.

Generally the male is smaller than the female. The genital orifice in the female is broad, slit-like and situated more posteriorly than the male orifice. The lips of the vulva protrude from the oval depression of the integument. In the male the aperture is smaller. It has the form of a strongly arched crescentic slit.

The life history is the same as in *O. savignyi*. Egg stage lasts 7-8 days. Nearly 150 eggs are laid in the first batch. Larvæ are motionless and unable to feed. They have no respiratory openings on the skin. Three or four days later the larva undergoes the first ecdysis. In the first nymph stage it is very active and readily feeds as soon as the mouth parts are sufficiently hard. The female has five nymph stages and the male four. The intervals between the different ecdyses are not uniform and are dependent on complete digestion of the preceding feed of blood.

A hungry tick will soon start to gorge itself with blood; the body swells up, after which the discharge of the transparent secretion from the coxal glands commences. The coxal fluid is secreted by all the nymph stages as well as by the adult. The secretion of the fluid ceases several minutes after the engorged tick falls off.

The productivity of the female is increased by large feeds, and several batches of eggs are laid provided opportunities exist for repeated blood feeds. Oviposition goes on slowly and generally lasts for 10 days.

The adult can remain alive for at least one year in captivity without a blood feed. It generally chooses the fingers and toes for biting. The bite is followed by a tingling sensation and in some cases vomiting and purging may follow.

Other important *Ornithodoros* species of ticks.

O. turicata Duges, found in Colombia and in Central Texas but not on Gulf Coast, breed in wild rodent nests or holes.

O. megnini Duges, the spinose ear tick, found on horses in India, probably introduced from America or South Africa where it has been recorded from the ears of cattle, sheep and goats (Sen, 1937).

O. lahorensis Neum. Common in northern India and Turkestan. It is identified mainly by the character of the tarsi and the integument. It is believed to be an important vector of relapsing fever in Central Asia.

O. crossi Brumpt. Cross and Patel (1921) reported successful transmission of the trypanosome of surra by the bites of *O. lahorensis* and *O. crossi* in the Punjab; this has, however, not been confirmed by Yorke and Macfie (1924).

Relapsing fever. The relapsing fever in Tropical Africa is caused by *Spirochæta duttoni* and the disease is carried from man to man by the human tick, *O. moubata* (Dutton and Todd, 1905). The tick serves as the true intermediate host for this disease and the infection is transmitted to their offspring (Koch, 1905).

The morphological differences between the various spirochætes are insignificant and they are almost entirely distinguished by their immunity reactions. Manteufel (1908) and Neumann (1909) have shown that *Spirochæta recurrentis* and *S. novyi* as well as *duttoni* may be transmitted by *O. moubata*.

The life history of the spirochæte in the tick is almost identical with that of European relapsing fever which develops in the louse. The spirochæta quickly disappear from the intestine of the tick and it is thought that they break up and enter the coelomic cavity where further development takes place. It has been supposed that the spirochæta escape with the coxal fluid by which infection of the wound takes place. Hindle (1911) failed to find them in the coxal fluid of infected *O. moubata*. In his opinion the infective material contained in the excrement is carried by the coxal fluid into the wound caused by the bite of the tick. On the other hand Kleine and Eckard (1913) detected the spirochæta in the ovaries and also in the coxal glands, Malpighian tubules, cephalic glands, stomach and salivary glands in infected *O. moubata*. Nicolle and Anderson (1926) later proved that the bite of the tick is not infective but that infection is caused by crushing the tick.

The part the coxal fluid plays in the mechanism of infection of relapsing fever by ticks has been definitely determined by Adler, Theodor and Schießer (1937) in *O. papillipes*. This tick lives in caves and acts as a natural carrier of relapsing fever in Palestine. It does not pass any coxal fluid while feeding. These authors are definitely of the opinion that infection can take place by the bite alone. The infection, however, passes through the eggs.

Although *O. savignyi* has been suspected of playing the same role as *O. moubata* in the carriage of relapsing fever spirochæte, Kirk (1938) failed in transmitting this disease through *O. savignyi*.

Other relapsing fever ticks are (a) *O. parkeri*, a vector in Utah and along the Colorado river in the United States; (b) *O. talaje* Guérin-Men. in Panama; (c) *O. turicata* Dugès. in Colombia; (d) *O. tholozani* (Lab-Meg.) in Persia; (e) *O. lahorensis* Neum. in Central Asia; (f) *O. papillipes* Bir. in Persia and Turkestan. The separate identity of this species as distinct from *O. tholozani* has not been finally settled though Desportes (in a private communication to the author) claims that *O. papillipes* in Turkestan, *O. tholozani* in Persia, *O. asperus* in Syria and *O. crossi* in India are closely allied, if not, they belong to the same species.

Dissection of Tick.

A gorged female should be starved till much of the ingested blood has been digested. Hold the tick gently between the thumb and index finger of the left hand, and with a razor make an incision at the lateral border of the abdomen. This is carried forwards as far as the head, and backwards round the posterior margin of the body. The ventral integument is now fixed by small pins to the paraffin block in the dissecting tray. Further dissections are performed in 4 per cent formalin solution under a dissecting microscope. The dorsal integument is now gently separated from the body by cutting with a knife-edged needle the tracheal and other attachments. As soon as the skin has been reflected forwards the intestine and the ovary which lies transversely, will come into view. The salivary glands are also easy to find.

Section cutting.

For section cutting hot Bles may be used. Robinson and Davidson (1913) found Carnoy's glacial acetic acid + absolute alcohol + chloroform mixture in proportions of 1:6:3 a very satisfactory fixative. Nevertheless on account of the cuticle being highly chitinised in ticks, serial sections are difficult to cut. Cowdry and Ham (1932) recommend the removal of the chitinous coverings by careful dissection after which complete serial sections can be easily obtained. According to Shortt (1936) the dorsal chitin should first be removed by dissections and sections should be cut in a tangential plane; this will not interfere with the chitin on the ventral surface.

Sen (1941), however, tested a large number of fixatives including those of Carnoy, Duboscq-Brasil, Bles, Bouin, Mukerji (1937) and Petrunkevitch, but the best results were obtained with Sherlock's fluid consisting of 95 parts of saturated solution of mercuric chloride and 5 parts of acetic acid (Eltringham, 1930). The advantage of the last named fixative is that it is free from alcohol, which has a hardening effect on the chitin. For dehydration, "solvax" is for the same reason substituted for alcohol. This substance has been claimed to be an effective dehydrating agent by its manufacturers, Messrs. Flatters and Garnett, Manchester, and has been reported to be highly satisfactory by Eltringham. "Solvax" will dehydrate rapidly from 20% alcohol and mixes freely with paraffin and xylol. It is considered to be an ideal medium for embedding purposes.

The method of procedure as advocated by Sen is as follows:—

1. Fix in Sherlock's fluid for 3 hours.
2. Wash in distilled water for 2 minutes and stain in a strong watery solution of eosin for 3 minutes, in order that the object may be easily seen while being imbedded in paraffin.
3. Transfer to 50 per cent alcohol for 48 hours, with iodine solution for the removal of mercuric crystals. Remove to "solvax" and leave the object for 48 hours, changing the fluid thrice.
4. Place in methyl benzoate celloidin solution (10 grammes of celloidin dissolved in 1,000 c.c. of methyl benzoate) for 48 hours, with two changes of the solution. Transfer to benzol for 24 hours, changing the fluid once. Place for 15 to 30 minutes in benzol-paraffin at 37°C. This represents Peterfi's methylbenzoate method.
5. Keep in melted paraffin (melting point 60°C.) for 1 hour.
6. Imbed in a watch-glass, orientating the object with warm needles under a magnifying glass.
7. With a glass pencil draw a line on the edge of the watch-glass to indicate the long axis of the object.
8. Plunge the watch-glass into cold water, and with a fine needle draw a line on the edge of the wax in continuation of the one previously drawn on the watch-glass in pencil. Using this line as a guide, the object may be cut in any direction, a suitable thickness for the sections being 8 to 10 microns.

The removal of the paraffin and celloidin from the sections should invariably be followed by a momentary immersion of the slides in a 0.8 per cent solution

of celloidin in ether-alcohol, as recommended by Guyer (1936). The omission of this step is likely to result in the sections coming off the slides while being passed through alcohols or washed in water.

Very good results have been reported with Mann's eosin-methyl-blue stain. By reason of the rapidity of its action, this stain will prove more advantageous than Heidenhain's iron hæmatoxylin.

Breeding.

For egg-laying, a gorged female should be kept in a glass tube on a bed of sand slightly moistened. A few twigs of (moistened) grass are also placed inside. After the tick has been introduced, a piece of cloth is tied round the open end of the tube. The larvæ when they have emerged from the eggs, should be fed on the ear of a rabbit, but should not be left on the rabbit.

Clearing and staining.

For clearing, a 10 per cent solution of potash is recommended. It should always be used cold. If necessary the cuticle can be stained with picric acid in xylene, fuchsine, Bismarck brown etc. before mounting in Canada balsam.

Collection and examination of ticks.

Ticks should be removed with forceps or tweezers. The danger of leaving the head of the tick embedded in the skin after the tick has been removed should be borne in mind. This can be avoided by killing the tick with pyrethrum, and pulling it off with forceps after 10 to 15 minutes. The pyrethrum may be used either in the liquid form as extract, or as an ointment prepared with the dry powder in vaseline. Ticks from one species of animal should be collected in separate tubes and preserved in 75 per cent alcohol.

For identification they should be dried between folds of blotting paper, suitably placed on plasticine, and examined under the microscope. In some cases slide preparations will be needed and the carbolic acid method, after the parts have been treated with caustic potash, will prove satisfactory.

Prevention.

A knowledge of the life history of the tick concerned is important. Particular distinction must be made between the tick of the host and the tick of the habitat. The former includes all hard ticks and the latter soft ticks. The hard ticks should not only be attacked on the host itself but also in the adjacent places where the animal moves about or takes shelter at night. While it is easy in the case of the dog tick, cattle ticks are particularly difficult to destroy for the reason that the female oviposits in the field where the animals graze. In such cases efforts must be concentrated in systematically destroying the ticks, especially the females found on the animal. In this way the propagation of the tick will be greatly limited though it will not be possible to eradicate the pest completely.

For all round purposes a solution of pyrethrum prepared in kerosene is very effective for destroying ticks by spraying. Pyrethrum is extremely toxic to ticks. It can also be used on dogs but where extensive ulceration of the skin is present, it should be used in the form of an ointment prepared with pyrethrum dust mixed

with vaseline in 8 per cent strength. The ointment should be rubbed on the tick. It is harmless to the eyes and even to the tender skin. If necessary it may be allowed to remain on the skin even for 24 hours.

The most practical and most extensively used method is dipping for three days in succession in an arsenical mixture which has the following composition.

(1) Arsenite of soda	4 lb.
Soft soap	3 lb.
Petroleum	1 gallon.
Water	400 gallons.
(2) Soda arsenate	1 lb.
Soft soap	$\frac{3}{4}$ lb.
Paraffin	2 lb.
Water	100 gallons.

The young ones are readily killed. If the gorged female is not dead, it may lay eggs but the eggs may not hatch; if they do, only a small number of larvæ are produced.

Dipping should be repeated every 8 days and may have to be continued for a year.

The dipping tank should have a capacity of about 2000 gallons of the fluid.

The removal of ticks by the process of dipping is not only rapid but also economical. From 400 to 600 heads of stock can be dipped in an hour at a cost of $\frac{1}{8}$ to $\frac{1}{4}$ of a penny per head. It must be remembered that the efficacy is entirely due to arsenic and when the arsenic content is fairly high, it will injure cattle. Too little arsenic, on the other hand, will fail to kill the tick. Evaporation will always tend to concentrate the mixture; it is for this reason that the dipping process is not popular with farmers. There is only a small margin between the efficiency of the liquid as a dip and the possibility of its doing harm to cattle. Further the solution of arsenite is liable to undergo oxidation when kept exposed in the dipping tank.

Conflicting views are held as to whether the tick absorbs the poison through its skin or imbibes it with the blood which it sucks from its host. Experiments tend to prove that the second view is the more correct one.

The North East of Scotland Sheep Tick Committee, 1938 recommend derris as a dipping fluid. The solution should contain 1 part of *Derris elliptica* root (containing 5 per cent rotenone) and 500 parts of water. This will give 100 per cent kill of ticks of all stages. Immersion for one minute is recommended.

It is much more difficult to destroy soft ticks as they lie buried in the soil or remain hidden in places where the fluid from the spray can not reach them. As far as possible fowl houses infested with ticks should be burned and the neighbouring ground should also be similarly treated by burning dried hay; otherwise a very powerful spray may have to be used. Where *O. moubata* is a pest, all holes in the walls and floor of mud huts should be sealed up with mud and cowdung mixed together.

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Family TROMBIDIIDÆ (Harvest or velvet mites)

These belong to the suborder Prostigmata in which the spiracles lie on the lower part of the basis capituli.

The typical members of this family are large bright red mites and from the velvety nature of their integument, they are known as velvet mites. They resemble the common harvest mites of Europe. The cephalothorax is well

demarcated from the rest of the body, which is densely covered with hairs. Eyes may be present or absent. The last joint of leg IV is not, or very slightly, shorter than the penultimate, and the last joint of leg I is usually swollen. The body is hairy. The palpi are 5-jointed, the penultimate one being provided with an accessory finger-like process. The legs are seven jointed.

Along the median line of the cephalothorax there is commonly a crista or dorsal groove.

The metamorphosis is incomplete. These mites in all stages are fluid feeders and generally feed on plant or fruit juice. Their red coloured larvæ are often found infesting beetles, bugs, spiders etc. In hot summers, especially during the harvest time, they may abound and may be exceedingly troublesome to man.

Genus *Trombicula* :

Eyes are absent ; crista enlarged at end ; pulvilli are absent.

Only two species are of medical importance as they transmit in their larval stages the virus of a typhus-like fever ; these are *Trombicula akamushi* (Japan) and *T. deliensis* (Sumatra, Java and India). The larval *T. akamushi* Brumpt is decidedly more reddish in colour than the larval *T. deliensis* Walch.

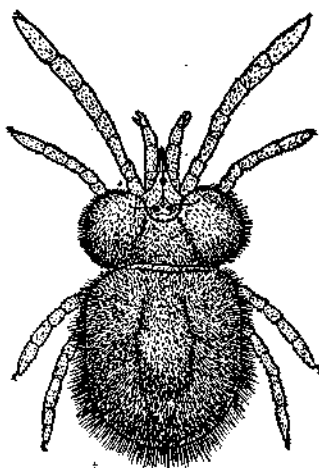


Fig. 143
Trombicula irritans.
(After Ewing.)



Fig. 144
A. *Trombicula akamushi*. (After Hirst.) B. *Trombicula deliensis*. (After Fletcher, Lesslar and Lewthwaite.) C. Scutum of *T. deliensis*. (After Fletcher et al.)

We owe it to Miyajima and Okumura (1917) for the exact information on the life history of *Trombicula akamushi*. This mite sucks blood only once during its larval life and it is so small that it is hardly visible to the naked eye. The parts of the body which are most often attacked by this pest are the scrotum,

the inguinal regions and axillæ. The larva remains on the host for three or four days and then drops to earth. In a week nymphs emerge. They are neither parasitic nor predacious, but can be reared on juicy vegetables, potatoes, melons, etc.; they will not eat any acid fruits such as apples and oranges. Moisture is essential for their growth. After growing somewhat they again seek shelter in the earth and a few days later assume a form almost like the adult but probably requiring another ecdysis. Both adults and nymphs are found under fallen leaves or decayed vegetation.

Of the harvest mites that are found in America and the Far Eastern territories which are known to attack man only 2 have been successfully bred out; these are *T. akamushi* Brumpt in Japan and *T. irritans* Riley in the United States. *T. hirsti* Sambon and *T. acuscutellaris* Walch have also been reported as attacking man in Malaya (Gater, 1932), and in Australia, *T. hirsti* Sambon and *T. australiensis* Hirst.

T. akamushi is found in great numbers within the ears of a field mouse, *Microstus montebelloi*, which acts as the reservoir of the virus. The larvæ are also found quite frequently around the rims of the eyes and the anus of rats. The mite does not convey the disease direct from rat to man. After it has fed once, it does not feed again but drops off from its infected host, and after passing through the nymphal phase becomes an adult. In these latter stages it feeds on decaying vegetable matter. The adult *Trombidium* lays eggs, and the larvæ, hatched from these contain the inherited virus acquired only during the larval stage and transmit it to the host on which they feed.

In Malaya a similar disease is believed to be transmitted by *T. deliensis* which are found on *Rattus rattus* and *R. concolor*; these rats are suspected to be the animal reservoirs. This view is supported by the observations that in some endemic areas, 10 per cent of wild rats give a well marked Weil-Felix reaction. There is no evidence that the human louse plays any part in the spread of the disease.

T. deliensis is widely prevalent on rats in India and is also thought to be responsible for the spread of the mite-borne typhus endemic in this country.

Any transmission experimental work with *T. deliensis* is not easy. The adult mites of both sexes can be obtained often in large numbers from the ears of field rats. They can also be obtained in smaller numbers from the earth around rat burrows. The presence of the mites is discovered by placing the suspected earth in a pan containing water when the mites will at once float on the surface. It is easy to induce the adult female to lay eggs from which larvæ readily emerge but the latter are difficult to recover after once they have been released on the ears of rats. It has not been possible to breed them in sufficient numbers beyond the larval stage.

Tsutsugamushi disease.

This is a specific infectious disease of the nature of typhus and is distributed principally along the course of the large rivers in Japan. It is transmitted by a mite known locally as *akamushi*. The etiological agent of this disease is

identified as a rickettsial form known as *R. orientalis* or *R. nipponica* (it has been named differently by different authors). The infection is transmitted to man from certain rodents and marsupials through the bites of various species of larval mites of the genus *Trombicula*. The larva feeds on blood only once and this is the parasitic stage during its whole life. Therefore the rickettsiae are carried through various stages of development and are transmitted through the eggs to the next generation and, according to Hayashi and Kato (1935), to a number of subsequent generations.

It must be borne in mind that infection contracted in the larval stage can only be transferred in the next larval stage.

There is evidence to suspect that other arthropods in addition to mites probably also act as vectors. However, mites appear to be the most important transmitting agent known at the present time.

Diseases similar to Tsutsugamushi disease have been described in Formosa, Sumatra, Philippines and the Malaya Peninsula. It has now been established that mite-borne typhus also exists in India.

In China, Formosa and Japan *T. akamushi* has been proved to be a carrier, whereas in Malaya, Sumatra and India *T. deliensis* is strongly suspected.

According to Walch (1923) the larvæ of *T. deliensis* have been found on *Acrocephalus orientalis*, a bird which has a migratory habit and may possibly carry the disease to distant countries. Keukenschrijver (1925) examined a number of crow-pheasants which frequent the undergrowth and jungle grass in Malaya, with the result that he found the majority of them harbouring *T. deliensis*.

Those who are interested in the habits and identification of *Trombicula* are advised to consult Walch (1924) and Walch and Keukenschrijver (1924).

Family TARSONEMIDÆ

The members of this family belong to the suborder Heterostigmata ; they are so-called on account of their possessing different forms of spiracles.

The Tarsonemidæ is a family of extremely small mites, which are mostly parasitic on plants and on other insects. In this family the spiracles are situated on the ventral surface just in front of the first pair of legs. The females are recognised by the presence of a club-shaped appendage, which projects between the bases of the first and second legs on either side of the body.

Genus *Pediculoides*.

In this genus the pedipalps are stout and curved and comprise almost the whole head.

The species *P. ventricosus* is so-called on account of the fact that in the pregnant female the lower part of the abdomen becomes swollen to form a large brood-sac inside which the embryos develop. These on hatching live within the mother till they are mature. They escape through the already thinned abdominal wall.

This mite is normally parasitic on insects and their larvæ and is found on straw and different kinds of grain in tropical countries. In the United States,

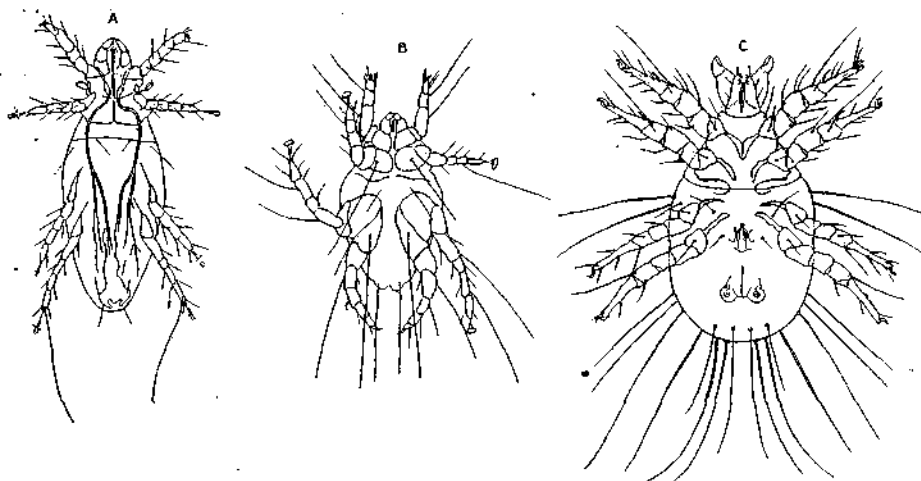


Fig. 145
A & B. *Pediculoides ventricosus*, female and male. C. *Tyroglyphus longior*, var. *castellani*. (After Castellani and Hirst.)

outbreaks of dermatitis acquired through sleeping on straw mattresses have been traced to this mite, while in England, O'Connor (1919) found the same condition in men engaged in unloading cotton seed from Egypt, and described it as being due to this species.

Family TYROGLYPHIDÆ (Forage mites)

The members of this family and also of Sarcoptidæ (itch-mites) have no external openings for respiration and are therefore included in the suborder Astigmata.

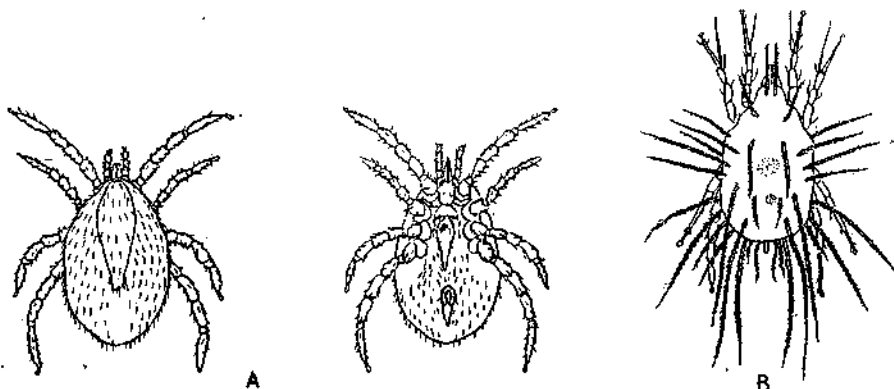


Fig. 146
A. *Liponyssus bacoti* (after Hirst), dorsal and ventral aspects. B. *Glycyphagus domesticus*. (After Hirst.)

Mites belonging to the family Tyroglyphidæ generally attack provisions and groceries. They are also found in decaying leaves. They are often found swarming in stores, and cause damage to sugar, flour, grain, cheese, cereals, dried fruits etc.

In this family there is a distinct cephalothorax and the chelicerae project like a rostrum. The body is elongate.

The life history of some of these mites is remarkable. Its growth from the egg stage takes place either in the normal way as occurs in other mites, *i.e.*, the larva is followed by a nymph which develops into the adult mite, or sometimes the nymphal stage may pass through another stage called *hypopus*. The hypopus is exceedingly small and does not feed. It is mainly adapted for the purpose of emigration by attaching itself to some active creature such as a fly or a mammal. As soon as it reaches the proper environment, it becomes detached and moults again into the nymphal stage and then turns into an adult.

The important genera which may concern the public health worker are:—

- (1) *Tyroglyphus*: the two common species are *T. longior* Gerv. and *T. siro* L.
- (2) *Glycyphagus*. *G. domesticus* de G.

Some of the hairs of the body of *Glycyphagus* are feathered and the claws of the legs are very inconspicuous, whereas in *Tyroglyphus* the opposite conditions are found. In *Tyroglyphus* the presence of a clavate or thickened hair on base of tarsi I and II and a suture between cephalothorax and abdomen will settle the diagnosis. Both may occasionally give rise to dermatitis.

Cheese on prolonged storage often becomes infested with *T. longior*. *T. longior* var. *castellani* are responsible for copra-itch contracted from handling infested copra. Water-itch or tea bush dermatitis is caused by *Rhizoglyphus parasiticus* Dalgetty, belonging to the family Tyroglyphidæ. The dermatitis usually occurs on the foot in tea-gardens during the rainy season (Castellani and Hirst, 1912).

Grocer's itch is a troublesome dermatitis for which *G. domesticus* is responsible.

Carter *et al.* (1944) have drawn attention to certain pathological conditions of the human lung supposed to be caused by mites. A large number of genera have been reported to be involved such as *Tyroglyphus*, *Carpoglyphus* and *Glycyphagus*, all belonging to the family Tyroglyphidæ, which also includes the well known cheese and sugar mites. Two species of *Tarsonemus*, a species of *Cheyletus* have also been found in human sputum. Subsequent to this, Soysa and Jayawardena (1945) have recorded similar findings in about 50 cases in a military hospital in the South-East Asia Command. All these cases were generally associated with a high eosinophilic count.

It has also been claimed that injections of arsenical drugs have brought about cure including the elimination of the animal parasites. How the latter are affected by arsenic when administered in the way stated above is difficult to explain.

Invasion of the human alimentary and urinary tracts by Tarsonemid and Tyroglyphid mites has been referred to by a large number of observers. Dickson (1921) claimed to have found *Tyroglyphus farinæ* in the trigone of the bladder.

Family SARCOPTIDÆ ✓
(Itch mites)

These cause itch in man and mange in domestic animals. All are parasitic. They are liable to attack both human beings and domesticated animals causing scabies and intolerable irritation. Mites belonging to Tyroglyphidæ and Sarcoptidæ have no respiratory openings and are therefore placed in the suborder Astigmata.

Genera of Sarcoptidæ.

(1) *Sarcoptes*: Anal opening is terminal; the dorsal surface of the body is provided with pointed scales and spines. These include the itch mite of man and of animals.

(2) *Notoedres*: Anal opening is dorsal; dorsal surface of the body with only short, sharp setæ. These produce similar diseases in cats and rats. It is much easier to find *Notoedres* than *Sarcoptes* from infested parts. (*Sarcoptes* and *Notoedres* are the true burrowing mites.)

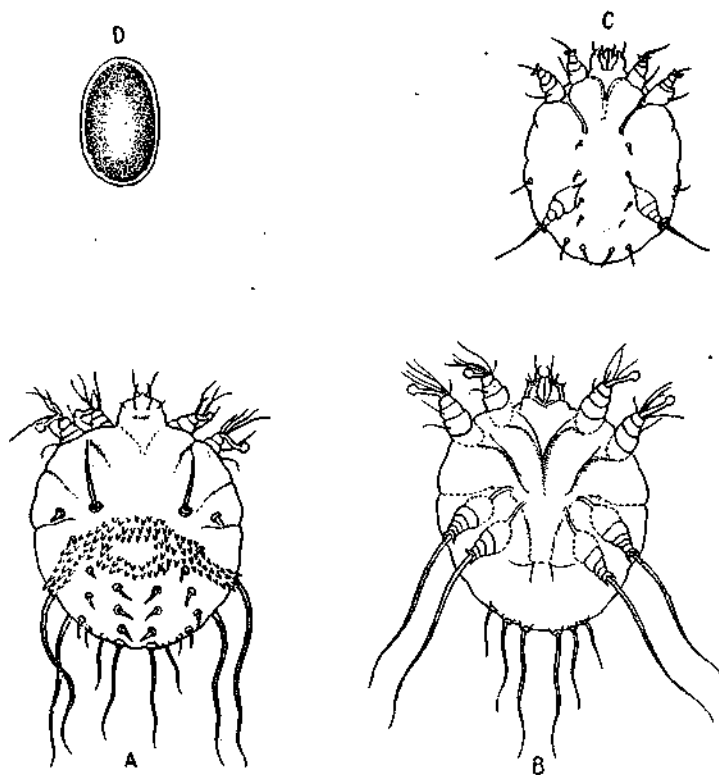


Fig. 147

A & B. *Sarcoptes scabiei* (female) from man; dorsal and ventral aspects.
C. Do; larva.
D. Do; egg.

(3) *Psoroptes*: Tarsal suckers with segmented pedicles. They produce scabs and do not burrow. *P. cuniculi* is responsible for extensive scab formation

in the ears, nose, face and even on the body of the rabbit. *Otodectes* produce similar diseases in dogs, sheep and cattle.

Genus *Sarcoptes*.

Two genera of burrowing mites are responsible for mammalian scabies, namely *Sarcoptes*, which affects man and larger animals, and *Notœdres*, which at times may affect man but is principally a parasite of smaller animals, such as cats and rodents. The genus *Sarcoptes* was formerly subdivided into numerous species, named according to the host upon which the parasite was found, but principally owing to the work of Warburton (1920) and Buxton (1921) these species are now generally accepted as varieties of a single species *S. scabiei*. Many of the species that are normally confined to one host can live on other animals and also on man.

Mites of the genus *Sarcoptes* are extremely small, globular in shape and of a whitish colour. There is no definite segmentation of the body though it is clearly divided into two regions by a fold of the integument. These two regions are generally called the notothorax and the notogaster.

The integument shows transverse striations and is provided with scales, hairs and spines. The legs are short and stumpy and are arranged into two groups. Each is provided with a powerful claw. Leg one and two terminate in ambulacra, long tubular processes ending in a bell-shaped sucker. In female mites these ambulacra are not found on legs 3 and 4, each of which is produced into a long bristle. In the male the fourth pair of legs terminate like the anterior legs in ambulacra, while the third has a long bristle. The first and second legs are similar in all respects. Both possess 5 joints. The third and fourth pair of legs are smaller and each consists of 4 joints. The anus is terminal.

Life history of *S. scabiei* var *humanus*.

The following is an account of the life history of the scabies mite of man described by Munro (1919).

The female burrows into the epidermis of the skin. The burrow is a fine winding line and is never deeper than the malpighian layer. The parts of the body which she selects for her burrowing and oviposition are well defined and are as follows:—The interdigital spaces, the wrists especially the ulnar margins, the elbows and anterior folds of the axillæ, the penis, scrotum and buttocks, the back of the knee and the ankles and toes.

The ovigerous female proceeds to form the egg burrows after the last moult. Under normal conditions the burrowing corresponds more or less to the time spent in bed, which is roughly eight hours.

Oviposition commences almost simultaneously with the burrowing. The number of eggs laid by the *Sarcoptes* of man according to Gerlach (1857) is more than 21 and may be between 40 and 50. The maximum number of eggs in one burrow as observed by Munro was 14.

The hatching time of the egg varies from 70 to 80 hours. Blanchard (1890) states that development may commence before oviposition and that the eggs are viviparous. The same thing was noticed by Munro in the case of sheep itch

mites. Ova have never been obtained from acari removed from the body of the host.

Munro experienced great difficulty in obtaining larvæ and nymphs even when numerous scabies cases were available. In searching for these stages he removed complete burrows and surrounding skin. This method yielded larvæ and on one occasion a first-stage nymph.

According to Munro larvæ are found in egg burrows and also in the scrapings of the skin in the neighbourhood of the burrows. They can also be obtained in the neighbourhood of isolated vesicles. The larvæ when released on the skin will bore into the hair follicles where a vesicle makes its appearance from which larvæ can be recovered.

According to the same author there are two nymphal stages though he was unable to obtain any specimen of the first nymphal stage. The second nymphal stage or immature female is found in burrows which are smaller and are unaccompanied by vesicles. Pockets or short blind branch burrows may be found opening into them, which are the work of the male. Mating occurs at this stage. After moulting in the burrow and leaving the cast skin in it, she turns into a mature female and proceeds to make the egg burrow proper.

The duration of the different stages as found by Munro is as follows:—

Egg	2½ to 3½ days.
Larva	1½ to 3 days.
First nymph	1½ to 2½ days.
Second nymph	2 to 4 days.

The whole life cycle from egg to egg covers a period of from 9 to 15 days.

Mellanby (1943) holds the view that both larvæ and nymphs enter hair follicles for shelter and food.

In our observations on patients and on volunteers artificially infected we did not come across a single male individual and only one specimen of a larva taken from a burrow which had just escaped from the egg shell was found. The rest were all nymphs of two sizes and mature females. In most cases the females were gravid. The largest number of eggs found by us in a burrow were two. We are therefore inclined to hold that at least in the tropics, the life history presents some variations from that found in temperate places. From what we have observed we are tempted to construct the following life history of *S. scabiei*.

The egg is ovo-viviparous and the larvæ on emerging from the egg-shell quickly escapes to the surface; it has a short life and it moults to form the nymph which digs into the skin for the first time. A papule appears at the spot selected by the nymph for burrowing. The appearance of the papule perhaps synchronises with the maturation of the nymph which provokes considerable irritation and this induces scratching with a view to enable the parasite to escape to the surface of the skin. At this stage the nymphs have attained two sizes, one small and the other large. Moulting takes place and the small-sized nymph turns into the male mite and the large-sized nymph into the female mite. The male has a short life and probably does not burrow.

Itching is the only symptom that is produced by the mite and for this reason the name 'itch' has been given to this disease. The parts of the body particularly selected by the mite for burrowing have already been stated. In a large number of cases it is possible to extract mature or ovigerous females from such situations. In a moderately severe case the chest, back, front of the abdomen, inner surfaces of the thighs, legs and arms become also the sites of troublesome itching. In these places nymphs are generally found. The disease even extends to the neck, cheek and back of the scalp, and from all these places nymphs have been recovered. Secondary infection with pyogenic cocci generally sets in but may not take place at all. When it occurs, it is generally confined to the hand, wrist, elbow, buttock, scrotum, penis, waist, and from the knee downwards though in a small number of cases other parts may also be involved.

The female after depositing the full complement of eggs dies at the blind end of the burrow.

In experimentally transmitted infestations Mellanby (1944) observed that itching is generally experienced at least a month after the introduction of the parasite; after six weeks the irritation is usually sufficient to cause disturbance of sleep. The appearance of this symptom so late after the entrance of the mite into the skin of the host has been considered by Mellanby as an indication that the symptoms are the result of sensitisation produced by the parasite. On the other hand we are inclined to hold from our observations that a large number of parasites are necessary to cause disturbance of sleep, or to attract the attention of the patient to the fact that he needs medical attention. If a large number of ovigerous females are introduced, marked symptoms appear within a week. It has been observed that the papules are associated with the most intense itching.

So-called severe cases of scabies showing well-marked clinical symptoms may have large mite populations. According to Johnson and Mellanby (1942) 63 per cent of the mites are found on the hands and wrists, 10.9 per cent on the elbows, 9.2 per cent on the feet, 8.4 per cent on genitals and 4 per cent on the buttocks. In the presence of secondary infection mites cannot thrive, and under such conditions it is difficult to find them.

The itching is much more intense at night than in the day time, and from this it was once believed that the symptoms are due to the mite being specially active at night. When either the female or the nymph burrows, it is not felt by the patient and therefore no satisfactory explanation of the cause of the itching experienced specially at night has been offered.

Transmission of scabies.

Unless it is a fertilised female the transfer of a single individual from a patient to a volunteer will lead to no results and eventually the parasite will die. But if the object is to induce an artificial infestation, either younger stages of both sexes must be transferred or better, a fertilised female. The transference of the latter is likely to lead to a rapid multiplication of the parasites in the body of the healthy host.

However, knowing the complete life history of the mite it is difficult to accept

the claim made by Johnson (1943) that he has been able to cure the infestation of patients by merely looking for adult females and removing them.

The problem of the extent to which mites leave their burrows and the degree to which scabies is capable of being transmitted by clothing, blanket etc., is a matter of considerable practical importance and one about which divergent views have been expressed. Mellanby (1941) shares the same views with Hebra (1868) that the possibility of the migration of mites from the body to clothing, bedding etc. is small. In fact Mellanby has found that prolonged contact, e.g., sleeping on the same bed, is necessary for the spread of the disease. On the other hand, Mac Cormac and Small (1917), Munro (1919), Lydon (1941) and the present author hold opposite views. According to them prophylactic measures, in addition to the treatment of the patient, should also include disinfestation of his personal belongings with which he comes into close contact.

Immunity.

When volunteers, who had already a generalised infection of scabies, are cured and then reinfested, there is always a marked local reaction. In the case of such secondary infection the mite population does not rise very high as generally occurs in primary infection. Mellanby noticed that this disappearance or reduction of the mite population is connected with an intense skin reaction (Mellanby, 1944).

Sarcoptes scabiei var. *scabiei-crustosae* Fürst.

Norwegian scabies or crusted scabies is a well-defined condition for which a mite resembling the ordinary scabies mite of man but presenting minor differences only in the size of the spines, has been thought to be responsible (Buxton, 1921).

In this disease the skin is considerably thickened over the affected parts which are more commonly the hands and feet and this is associated with the formation of thick crusts in which enormous numbers of *Sarcoptes* of all stages are found. It has been proved that these mites are also capable of burrowing in the normal way. The condition is not uncommon in the tropics but instead of all stages of *Sarcoptes* being found we have never encountered any male or larval mite.

This variety of scabies is at present considered to be in no way different from the ordinary type and according to us it is found only in long standing cases and not necessarily in dirty and unclean individuals.

Sarcoptocidal drugs.

(1) Sulphur. Though sulphur has no direct effect on the mite, yet when it is properly applied on the body, all the mites will be killed. To obtain the best results the sulphur should be used mixed with vaseline or better with soft soap and applied over the whole body for three days in succession. The patient should properly clean his body once before, and again at the termination of the treatment but should desist from taking any bath during the intervening period. This treatment is extremely unpleasant to follow and very often leads to a severe type of dermatitis. On account of these reasons it is not very popular, especially in tropical conditions.

(2) Benzyl benzoate. This is an important ingredient of balsam of Peru which has been in use in the treatment of scabies for a long time. Benzyl benzoate was

introduced for the treatment of scabies by Kissmeyer (1937). It is used as an emulsion (benzyl benzoate, 20% with an equal quantity of alcohol and soft soap). Benzyl benzoate is directly sarcopticidal and though the proper opening up of the mite burrows is strongly recommended before it is applied, it has been found that a preliminary bath can be dispensed with but should be taken afterwards. (Mellanby etc., 1942). On account of the presence of alcohol it causes a burning sensation which does not last more than a minute. Two applications on consecutive nights will practically cure the disease.

(3) Gordon and Seaton (1941, 1942), after making comparative studies with (1) Dimethylthianthrene, (2) Tetraethylthiuram monosulphide ("tetmos"), and (3) Benzyl benzoate, found "tetmos" as efficient as benzyl benzoate in killing *Notoedres* mites but as it has not the same action on eggs, the treatment will have to be repeated after an interval of 4 days.

Davey and his collaborators (1944) have demonstrated the curative effect of soap impregnated with "tetmos" once weekly for two to four consecutive weeks and Gordon and Unsworth (1944) have drawn attention to its prophylactic effect on infection with scabies due to *Notoedres*.

(4) Roy and Ghosh (1944) found oil of turpentine to have the same action on mites as benzyl benzoate, and later trials on patients with this drug have met with promising results. It is prepared as follows:—

Oil of turpentine B. P.	...	20 parts.
Soft soap or soap shavings (bar soap)		q.s.
70 per cent alcohol	...	80 parts.

Soften the soap with water. Mix the oil thoroughly with the soap in a mortar with a pestle. Mix the spirit gradually.

When alcohol of a higher strength, e.g., bazaar methylated spirit is used, it has the advantage that the emulsion dries up from the skin very quickly. Turpentine when mixed with oil has no action on the mite and it must therefore be used with spirit alone.

A preliminary cleansing of the skin with soap and hot water before the application of turpentine emulsion on the skin is not essential. Neither is it necessary that bath should be taken immediately afterwards. It may be taken after half-an-hour after the emulsion has been applied or may even be postponed till the next day.

(5) Rotenone. As it is likely to produce severe dermatitis especially in the scrotal region of the body, it cannot be safely used.

(6) Pyrethrum (*P. cinerariaefolium*). Pyrethrum itself has no action on mites as the latter respire through the cuticle and the insecticide enters the body of an insect through the respiratory opening (Roy and Ghosh, 1944). It has, however, a marked effect on impetigo, i.e., when secondary infection has set in. An ointment prepared with the dust in 10 per cent strength when applied to the sores will cause the pain and swelling to subside generally within 3-5 days.

Animal Mange.

Ear mange of rabbits is most frequently caused by *Psoroptes cuniculi*, and

sometimes by *Sarcoptes cuniculi*. The infection may penetrate to the middle ear, or even to the inner ear and in exceptionally severe cases may even cause death.

Skin mange in rabbits is produced by *Sarcoptes scabiei* var. *cuniculi* and *Notoedres cuniculi* which occurs on the nose, lips etc. and may spread to other parts of the body. Sarcoptic mange spreads very rapidly and is fatal in a few weeks if not checked. However, this type of mange is rare in the rabbit, in which the usual form is caused by *Notoedres cati* var. *cuniculi*.

Mange in horses is due to *Sarcoptes scabiei* var. *equi*, *Psoroptes moccunus* var. *equi*.

Scabies or mange in cattle is a specific disease of the skin caused by *Psoroptes communis* var. *bovis*. In sheep *Otodectes* produce the same type of disease.

Notoedres cati and *N. muris* affect cats and rats respectively. In cats, the ears, forehead and neck are the parts principally affected.

Mange in animals quickly responds to sulphur treatment, also to pyrethrum ointment used in 8 per cent strength but recurrence is likely to occur unless the treated animal is separated from the rest.

Family Gamasidæ (Insect and rat-mites)

The species of mites belonging to this family have the respiratory openings between the coxæ of the third and fourth pairs of legs and are placed in the suborder Metastigmata.

The majority of the Gamasidæ are free-living, only a few being parasitic. There has been a tendency to include the parasitic species in one separate family Dermanyssidæ which are found on birds, reptiles, small mammals, bats and small rodents.

The important characters of Dermanyssidæ are the following: The fingers of the chelicerae of the female are short, stout and armed with distinct teeth; body covered both above and below with chitinous plates; anal plate nearly always present and distinct from the ventral plate in females; the genital opening in the female never completely surrounded by sternal plate.

Among those found on rats the following genera are important. (1) *Liponyssus*, (2) *Dermanyssus* and (3) *Laelaps*.

Genus *Liponyssus*. The chelicerae are slender and pointed.

L. bursa Bert., the tropical fowl mite, occasionally attacks man. *L. bacoti* Hirst also attacks man and can transmit endemic typhus from rat to rat.

Those who live in close proximity to pigeons are known to be bitten by these mites. Three such cases, all women, came to our notice. They had papules on the body which were at first thought to be due to scabies but it was later discovered that the condition was entirely caused by bites of *L. bursa*. The results of precipitation tests clearly proved that the ingested blood was derived from man.

L. bacoti Hirst. This mite is normally found on rats and is known as the tropical rat-mite. It also readily attacks man and is a prolific breeder. It h

been suspected of transmitting endemic typhus from rat to rat, and on epidemiological grounds alone it is believed to be a carrier of this disease in Texas. Smith and Mehta (1937), however, found that this species is unable to take up and develop the virus of typhus of the X 19 type.

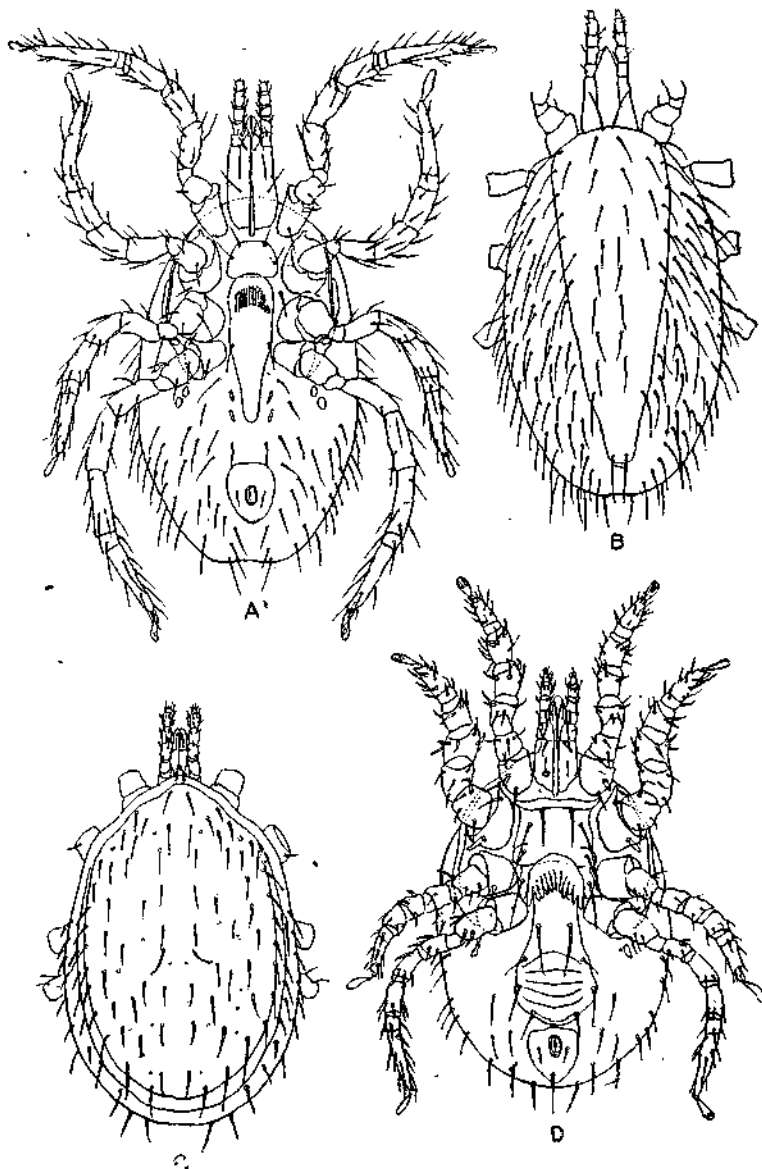


Fig. 148

A & B. *Dermanyssus muris*, (from Hirst) ventral and dorsal aspects.
C & D. *Laelaps nuttalli* (from Hirst); Dorsal and ventral aspects.

Genus *Dermanyssus*. It is essentially a chicken mite. In this genus the chelicerae are united to form a very long style. Various cases of *D. avium* attacking human beings have been reported.

Genus *Laelaps*. The fingers of the chelicerae of the female are rather short, fairly stout and armed with distinct teeth.

Key to the principal genera of blood-sucking mites on rats. (Hirst, 1926.)

- | | | | |
|--|-----|-----|---|
| 1. Fingers of mandibles (Chelicerae) in female sex fused to form a very long and slender style | ... | ... | <i>Dermanyssus</i> . |
| Fingers of mandible short in female sex | ... | ... | 2 |
| 2. Fingers of mandible of female very slender and without teeth | ... | ... | 3 |
| Fingers of mandible of female usually stouter and with distinct teeth | ... | ... | 4 |
| 3. Anal plate of female very large and not pear-shaped | ... | ... | <i>Myonyssus</i> . |
| Anal plate of female quite small and pear-shaped | ... | ... | <i>Liponyssus</i> . |
| 4. Genito-ventral plate of female furnished with numerous hairs. Anal plate often with more than 3 hairs | ... | ... | <i>Hæmogamasus</i> . |
| Genito-ventral plate of female with only a few hairs | ... | ... | 5 |
| Hairs on anal plate only three in number | ... | ... | 6 |
| 5. Four pairs of hairs on genito-ventral plate of female | ... | ... | <i>Laelaps</i> . |
| Only one pair of hairs on genito-ventral plate of female | ... | ... | 6 |
| 6. Second leg very stout and furnished with strong spines, its femur with a strong ventral spur | ... | ... | <i>Androlaelaps</i> . |
| Second leg usually not so stout, its femur without any strong ventral spur | ... | ... | <i>Hypoaspis</i> .
(<i>Hæmolaelaps</i>). |



Fig. 149
Demodex
folliculorum.
(After

Family Demodicidae (Follicle mite)

They are elongate and worm-like. The posterior extremity is tapering. They have no respiratory openings and the respiration takes place through the cuticle. They are parasitic.

D. folliculorum Simon. While the dog follicle mite, *D. canis* Leydig, causes a severe acariasis in the dog known as red mange, the follicle mite, *D. follicularum*, is generally considered harmless. It is supposed to be a common parasite of man in all parts of the globe. It attacks the hair follicle and sebaceous glands producing no symptoms. At times their agglomeration in the sebaceous and meibomian glands may set up local inflammation (Braun, 1906). Africa (1933) has reported a case of pinhead vesicular eruptions along the borders of some old scars on the face, causing intolerable itching especially at night; this was associated with the presence of *D. folliculorum*.

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Order ARANEIDA ✓ (Spider).

In spiders, the constriction between the cephalothorax and the abdomen is very marked. The cephalothorax carries the usual mouth appendages, and on the ventral surface four pairs of legs. A large number of eyes are placed in two rows on the carapace of the cephalothorax.

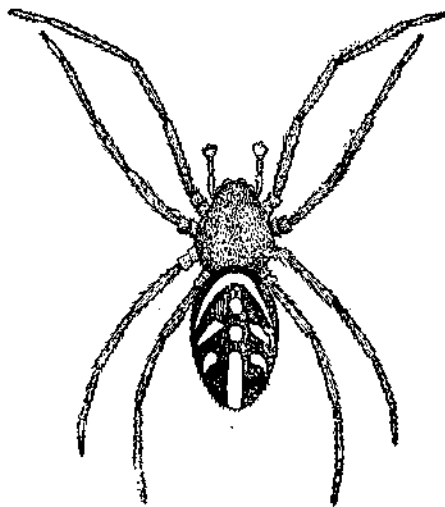


Fig. 150
Latrodectus mactans, male. (After Chamberlin and Ivie).

The first pair of appendages or chelicerae consist of two pieces; the basal piece contains the poison-gland and at the tip lies the curved claw or the poison fang. The poison is used for killing its prey. The spider like other members of class Arachnida is a fluid feeder and feeds on small insects which are caught in its web. The spider sucks only the juice of the insect and the carcass is left behind.

The body is clothed in hairs. They are all oviparous. Though they live mostly on land, there are some that are found in water.

The Araneida have been divided into two suborders and out of them

Opisthothelæ contains all the important species.

The venom of some spiders has been reported to possess marked toxic properties. The principal one among them belongs to the genus *Latrodectus*. This genus is widely distributed in North and South America, Europe, Africa

Palestine and Oceania. *L. mactans* is characterised by red spots which almost surround the anal orifice for which the name "hour glass" spider has been given to it. It is also popularly known as "black widow" spider. It is very dark in colour. The male is very small about half the size of a female which is not more than half-an-inch in length. The female has about three unequal red spots along the median line on the dorsum of the body. In the male the red spots are paler and in addition to the median spots found in females there are light-coloured transverse spots one each side of the median line on the dorsum of the abdomen.

The webs of *Latrodectus* spiders may be found everywhere. One common site especially in the rural districts is in privies just under the seats.

Majority of cases of spider bite occur on the genitalia both in male and female individuals at the time of defecation. Male spiders seldom cause any trouble whereas the females are highly poisonous. The bite causes sharp pain which soon becomes duller but extends to other parts of the body. There is profuse perspiration, the abdomen becomes board-like, speech is difficult and breathing spasmodic. Recovery is the rule though fatalities have been reported.

Among the three species of *Latrodectus* that are common in both northern and southern America, *L. mactans* is the most widely distributed and is the most feared.

It has been stated by Escomel (1919) that the toxic properties also exist in the eggs in more active forms in the fresh state than in the mature state. The venom contains a neuro-muscular toxin, a thrombokynase, a hæmolysin, and in the egg a proteolysin.

L. hasselti is found in Australia, New Zealand and Philippines. Its bite is also extremely painful and distressing.

In India the following two spiders have been investigated and none of them has been found to be poisonous to white mice and rabbits. These spiders are of large size and it is widely believed that their bites are followed by profound symptoms. These are *Poecilotheria miranda* Pocock, and *Olios punctipes* Simon.

Treatment.

Local treatment is of no avail as extirpation of the affected part within a few minutes after bite has no effect. The poison is absorbed very rapidly.

For relieving pain and for counteracting shock, morphia is ideal in cases where antiserum can not be used. An injection of antiserum (prepared by Mulford) in 5 c.c. dose and given intramuscularly will bring about relief of the symptoms within 15 minutes to half-an-hour. The antiserum is prepared by injecting an extract of cephalothorax of the spider in a horse and given daily for at least a fortnight.

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Order SCORPIONIDEA

Scorpions are prevalent over a large part of the globe and in India they are particularly more common in certain parts, e.g., in Central Provinces and Rajputana, than in others. At times they are found congregated together. They are viviparous and give birth to a large number of young ones at a time. The young ones resemble the parents.

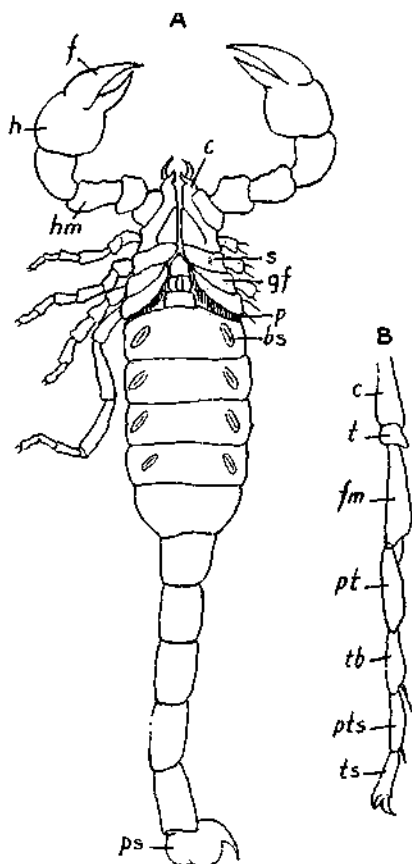


Fig. 151

A. Ventral view of scorpion.
bs, breathing slits; c, chelicera;
f, finger; gf, genital flap; h, hand;
hm, humerus; p, pecten; ps, poison sac;
s, sternum.

B. Leg of scorpion.
c, coxa; fm, femur; pt, patella; pts, pro-
tarsus; t, trochanter; tb, tibia;
ts, tarsus.

Scorpions are found in houses in dry and dusty places which are seldom disturbed. In hilly places they lie hidden in stones.

The body consists of cephalothorax and abdomen. The cephalothorax bears the two pairs of mouth appendages and four pairs of walking legs. The pedipalps are 7 jointed structures, the last two pieces forming the pincers for catching and holding prey. On the upper surface of the cephalothorax are placed two sets of simple eyes. There are a pair of large eyes close to the median line and a group of smaller ones on either side.

The abdomen is composed of 7 segments. On the ventral surface of the first segment lies a pair of rounded plates one on either side of the middle line which covers the opening of the genital ducts and is called operculum. The second abdominal segment bears the comb-like appendages called pectines. From the 3rd to the 6th are the openings of the lung books, one pair on each segment.

The tail which is attached to the sixth abdominal segment consists of six segments, the last one bears the pointed sting. Within this lies a pair of poison glands, the ducts of which open below the point of the sting.

The scorpion feeds on flies and other insects including cockroach. The prey is held firmly with the help of the pedipalps and is first stung and poisoned before

the juice is sucked. The carcass is left behind.

Scorpion eggs develop in the oviduct and when born, the baby scorpion even in size does not differ from the parent in external appearance.

Family Buthidæ: Sternum triangular ; in a few species it is pentagonal and in these species the last pair, or last two pairs of legs have tibial spurs.

Genus *Buthus*. Sternum triangular, carapace conspicuously keeled.

B. tamulus Fab.: 2nd and 3rd segment of tail distinctly larger than wide ; pectinal teeth 28 or more. Widely distributed in the Ethiopian Region, China, India but absent from Ceylon and Burma.

Fam. Scorpionidæ: No distal spurs ; claws of the legs overlapped by the sides of the tarsus.

Genus *Palamnaeus*.

P. fulvipes Koch: Legs reddish yellow ; humerus of pedipalps smooth.

P. swamerdami Simon: Humerus coarsely granular on the under side at the base.

Poisonous effects.

The venom of scorpions is tasteless, odourless, and colourless. When dried, the venom breaks into minute iridescent flakes.

The sting is followed by a sharp burning pain which is followed by throbbing and dull pain. The burning pain is confined locally. The affected part becomes red and swollen. In some cases there is cold perspiration and glycosuria. These effects generally pass off within 3 to 6 hours.

Experimentally the scorpion venom stimulates the respiratory centre, increases cardiac activity, raises blood pressure, stimulates the involuntary muscular tissue, activates the secretory glands and finally destroys life by its toxic action on the respiratory centre and on the nerve endings in the various parts of the body.

Snake venom produces the same effects, but the stage of stimulation is of short duration and the toxic symptoms are much more pronounced from the very moment they make their appearance. Snake antivenine has been found to neutralise the effects of scorpion venom.

Antiscorpion serum. Antisera for the venom of three Egyptian scorpions have been prepared. The antivenom has proved capable of neutralising the venom when mixed *in vitro* and also has proved useful both prophylactically and curatively in animals. It has a very marked effect on the intense pain following the sting (Todd, 1909).

Similar antivenom has been prepared against the poison of *Tityus bahiensis* and *T. serrulatus* in Brazil.

Caius and Mhaskar (1932), who have worked out the physiological actions of the venom of Indian scorpions, are of the opinion that even the largest species of scorpion of this country is unable to cause the death of a child though marked symptoms may be produced. Basu (1939) reported deaths from scorpion sting but these were probably due to snake bite.

Treatment.

An injection of morphia quickly relieves the pain and counteracts the shock. Local injection of novocaine will also relieve the pain quickly.

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Order PENTASTOMIDA
(Tongue worms).

They bear considerable resemblance to Arachnids although they possess annulations but these are only superficial. They are devoid of antennæ. The only claim to their being placed under Arthropoda is the presence of mouth hooks which represent the only remnants of appendages or limbs. All live parasitic lives suck blood in different stages of their post-embryonic existence.

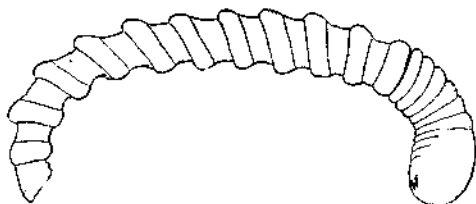


Fig. 152
Armillifer armillatus, male. (After Sambon).

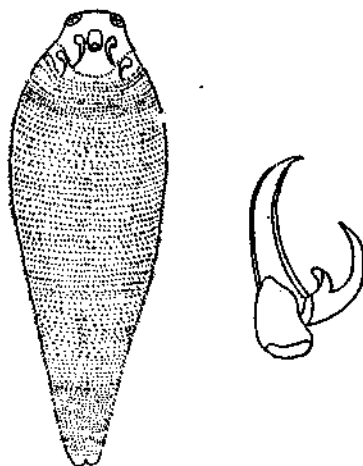


Fig. 153
Linguatula serrata (nymph) and
oral hook.
(After Roy and Ganguli).

The body is flat and worm-like. The mouth is distinct. Two pairs of retractile hooks encircle the mouth. The sexes are separate. All are oviparous. The larval and adult lives are generally passed in two different types of host, one herbivorous and the other carnivorous. The life history is imperfectly known.

There are only two important genera which are pathogenic to man.

Genus *Linguatula*.

The body is attenuated posteriorly. Hooks are simple, equal and disposed archwise. They are parasitic in mammals.

The adult is found in domestic animals of cosmopolitan distributions and the nymphal form has been reported from cattle, rodents and ungulates.

In *L. serrata* the eggs are passed in the discharge from the mouth and nostrils ; they also occur in the faeces ; when swallowed by the intermediate host the egg-shell is dissolved and the larva is liberated ; the larva then bores its way to the liver, spleen, etc. and after a series of moults it encysts, grows and becomes a nymph. When the nymph is swallowed by the final host, it makes its way from the gut to the lungs or nasal cavities where it becomes adult ; the nymph, when fully developed, is said to be able to leave the cyst and migrate to the bronchi or to the intestine of the intermediate host, from which positions it is passed to the exterior. It reaches its final host by being sniffed up by the dog and becomes adult in the nasal cavities (Southwell, 1924)

Both larvæ and nymphs have been recorded from man at autopsy and usually lodged in the liver. The adult is also known as a human parasite but this condition is very rarely met with and appears to be due to embryos wandering into the nose and developing directly. Among the domestic animals the dog is most frequently attacked and it acts as a definitive host ; the adult is lodged in the nasal and ethmoidal sinuses and also in the maxillary antrum.

Genus *Armillifer*. (Porocephalus): Body elongate and cylindrical and definitely ringed.

A. moniliformis. Occurs in the Oriental Region inhabiting in its adult stage the respiratory tracts of Indian pythons.

A. armillatus. These belong to the Ethiopian Region inhabiting the pythons of Africa and other snakes. They are known to attack man in Africa, both adults and larvæ being found encysted in the liver.

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Class MYRIAPODA (Centipedes and millipedes).

These are tracheate Arthropoda. The head bears many-jointed antennæ, a pair of eyes, and 2 or 3 pairs of jaws. The body consists of a number of similar segments, each bearing either one pair of legs or two pairs.

The tracheæ open by a series of stigmata usually in considerable numbers on the sides and lower surfaces of the segments.

This class has been grouped into two sub-classes, Diplopoda and Chilopoda.

Subclass Diplopoda (Millipede).

These are commonly known as millipedes. The genital aperture is situated far towards the anterior end of the body. With

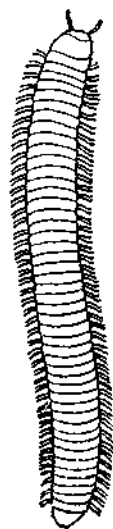


Fig. 154.
Millipede.

the exception of the first four, each segment bears two pairs of legs and represents two united true segments. There is only one pair of mandibles and one pair of maxillæ. They live on vegetable food. The commonest genus is *Julus*.

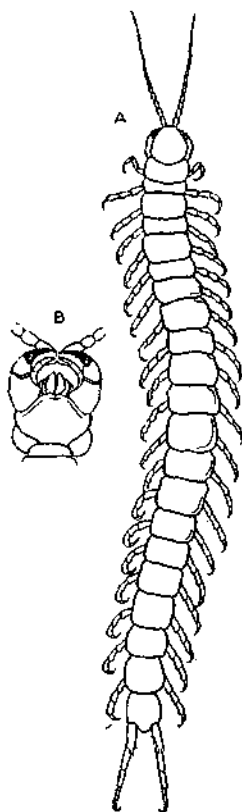


Fig. 155

A. Chilopoda.

B. Ventral view of head.

Subclass Chilopoda (Centipede).

The genital aperture in centipedes is situated at the posterior extremity of the body.

In Chilopoda the first pair of legs of the trunk is specially modified to act as maxillipedes or poison-jaws by means of which the centipede inflicts its poisonous bite. The segments are all dorsoventrally compressed. Each segment bears a pair of jointed legs. At the extremity of the pointed terminal joint of the maxillipede opens the duct of the poison gland.

It feeds on small insects and is oviparous.

Cornwall (1916) in an account of three Indian species stated from experiments that the venom is little toxic to laboratory animals. The bite no doubt is extremely painful and the pain lasts for 4 to 6 hours. In children other symptoms may supervene, such as nausea, vomiting, diarrhoea, glycosuria etc.

Death from centipede bite is rare though it has been reported by Coffin (1919) from Southern India.

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Class CRUSTACEA (Cyclops or water-fleas).

This class includes a very large number of animals of a diverse character and comprises crabs, lobsters, prawns, shrimps, wood-lice etc. With few exceptions, they live in water. They are abundant in all parts of the world and are generally free-living. They breathe by gills or cutaneously. The head always carries two pairs of antennae by which this class can be readily distinguished from others. Other appendages of the head consist of 3 pairs of jaws. The chitin is strongly calcified in a majority of crustaceæ. The sexes are separate.

Most of these creatures are unimportant to the medical officer who is concerned with only a few, especially the cyclops or water-fleas.

Subclass Copepoda

These are elongated Crustacea usually with distinct segments. There are five pairs of biramous thoracic appendages, but the last may be absent. The abdomen is without limbs. The females carry the eggs in external ovisacs.

They are widely distributed and are found essentially in fresh water. Some are free-living while others are ectoparasites especially on fishes.

Genus *Cyclops*.

These are minute creatures and are typical examples of free-living copepods. They are popularly known as water-fleas on account of their characteristic jerky movements while swimming. They are semi-transparent and though they are visible to the naked eye, their structure can only be studied under the microscope.

The body consists of an anterior broad mass which is called the cephalothorax, and an elongated posterior part, the tail or the abdomen. The anterior mass includes the head with which is fused the first thoracic segment. Its appendages are (a) a pair of antennules, (b) a pair of antennæ, (c) a pair of mandibles, (d) a pair of first maxillæ, and (e) a pair of second maxillæ, the last three comprising the gnathites or mouth organs. Antennules are very large and each consists of a large number of joints. These are the principal organs of locomotion. In the male they are peculiarly modified at the joints for holding the female during copulation. The antennæ are much smaller. Between the insertions of the antennæ in the middorsal line lies the small pigmented eye. The mouth is situated ventrally. Behind the cephalothorax lie 5 thoracic segments. Ventrally the first four thoracic segments bear swimming feet; the 5th segment bears a pair of vestigial limbs. The thoracic legs are united across the mid line with those of the opposite side. The last thoracic segment bears the genital aperture and is fused in the female with the first abdominal segment.

The abdomen or the tail may consist of 5 distinct segments which are devoid of any appendage. The last segment branches into two feathered filaments. The eggs are carried in external ovisacs attached to the first abdominal segment.

The alimentary canal is a narrow tube which runs from the mouth to the anus, the latter being located at the site where the last abdominal segment forks. The classification is based on the segmentation of the body and certain secondary sexual characters.

The growth of cyclops takes place by gradual stages. From the egg emerges the young larva or *nauplius*. It is extremely small and is provided with 3 appendages which grow into future antennæ and mandibles. The "nauplius eye" persists throughout its life. In the course of a few days the larva moults many times and is transformed into the adult cyclops.

The eggs of cyclops can resist desiccation and are liable to be blown about by wind. In this way fresh water previously free from cyclops may become infested.

Cyclops exist almost all over the globe. They are found not only in clean but also in muddy water. Some prefer stagnant pools, tanks, ditches, while some may be found in moderately flowing water. They all feed on solids such as diatoms, spores of aquatic plants, minute insects, etc.

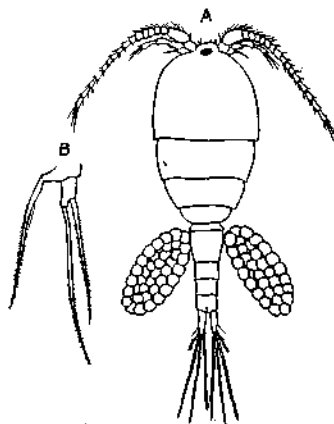


Fig. 156
A. *Cyclops leuckarti*.
B. Fifth foot of *C. leuckarti*.
(After Ward and Whipple).

In order to discover the existence of cyclops in a ditch or well, a net of the pattern of a butterfly or a fly net (the net being replaced by muslin), is dipped in water and when lifted, the water will quickly drain out through the cloth. The presence of cyclops is noticed as soon as the net is immersed in a bowl of water. It may be necessary to collect a small sample in a test tube and when held against a dark back ground, the cyclops can be quickly recognised by the characteristic jerky movement.

Mesocyclops leuckarti and *M. hyalinus*, both of which have wide distributions, are the two important species from the point of view of disease relationship and their distinguishing features are shown below.

	<i>Mesocyclops leuckarti</i>	<i>Mesocyclops hyalinus</i>
Male	Length 0.8 mm.	Smaller than female.
Female	Length 0.9 to 1.3 mm.	0.8 to 0.9 mm.
1st antenna	Long., has 17 segments, reaches the end of 3rd thoracic segment; only the last joint showing a hyaline membrane and an upper notch.	12 segments, reaches the end of 2nd thoracic segment; last two joints have hyaline membrane.
Receptaculum seminis	Characteristic: nearly as broad as long.	It is transversely very broad.
Egg sacs	Big, standing on the abdomen; contains 30-40 eggs.	Much smaller and contains only 6 eggs.
Distribution	Found universally in all waters: Europe, America, Asia, Africa, Australia.	Common in Europe, Africa, Asia and Central America.

Relation to Disease.

While a large number of species of cyclops exist, only two *Mesocyclops leuckarti* and *Mesocyclops hyalinus*, are known to act as intermediate hosts of guinea-worm, *Dracunculus medinensis*. The adult female worm, which is the only sex known, attains a length of 4 to 5 feet and is a connective tissue parasite of man which comes to rest under the skin of feet, legs, arms and occasionally the back. A blister appears on the skin just at the site overlapping the vagina which generally protrudes through a small rent when the skin of the blister gives

way. Millions of embryos are discharged in a milky fluid through the opening. A large majority of the embryos are killed and it is only when they reach the suitable species of cyclops in a tank, well or pond that development proceeds. The infective stage is reached in the arthropod host in about 8 weeks time.

According to Leiper (1906) the embryos enter the cyclops by way of the alimentary tract, a view also shared by Roubaud (1913). The larvae of guinea worm actually reach the body cavity in from 1 to 6 hours after being swallowed (Moorthy, 1938 ; Moorthy and Sweet, 1938). Proof has also been adduced in favour of certain species of cyclops also acting as intermediate hosts of the tape-worm *Diphyllobothrium latum*.

It has been found by Moorthy (1932) that healthy cyclops live longer than infected ones which may live as long as 62 days. On the other hand Southwell and Kirshner (1938) have shown that even when 15 larvae are present, the cyclops is not affected in any way. The guinea-worm larva undergoes certain changes inside the body of the cyclops such as increase in size and casting off of its tail. Two ecdyses occur. The guinea-worm larva may live in water for 7 days. Both guinea-worm and cyclops die in brackish water.

In nature the infection is found in the younger forms of both sexes and none in those bearing ovisacs.

The influence of acid on cyclops infected with guinea-worm larvae was first demonstrated by Leiper (1906). He showed that 0.2 per cent of hydrochloric acid which approximates the strength of acid in the gastric juice, kill the cyclops and activate the resting guinea-worm larvae within its body cavity. The larvae attempt to escape from the body of the cyclops. They generally escape by breaking through the cuticle. Even a lower concentration of acid, much below what is assumed to be necessary, is sufficient to kill the cyclops (Rao, 1936).

Although cyclops are found in a large variety of water, step-wells and ponds are considered to be the most important sources of infection to man in India and on the west coast of Africa.

Diphyllobothrium latum.

Besides man, the infection has been reported from domestic animals such as dogs and cats. The eggs are discharged in water where they hatch and produce infection in the cyclops in the same way as the guinea-worm. Later fish become infected by swallowing infected cyclops. The species of cyclops involved is not known.

Gnathostoma infection.

Cyclops is also an intermediate host of *Gnathostoma spinigerum* producing gnathostomiasis in man and animals. The exact mechanism of infection is not known but it is thought that man becomes infected by eating uncooked or improperly cooked fish already infected by eating infected cyclops.

Prophylaxis.

Prophylaxis of guinea-worm is easy if it is remembered that man becomes infected by drinking water from step-wells which harbour infected cyclops. The main object in the prevention of the disease is either to destroy the cyclops or pre-

vent them from finding access to the stomach of man. The following measures are therefore recommended.

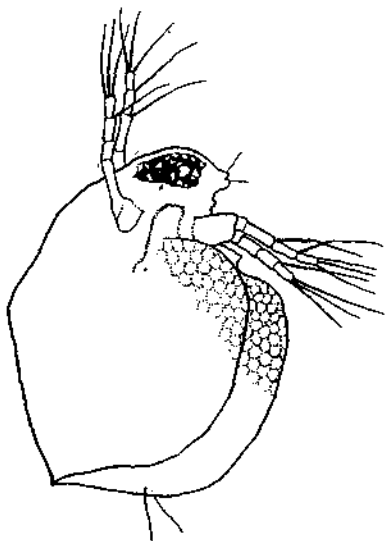


Fig. 157
Daphnia.

- (a) Conversion of the step-wells into draw wells.
- (b) Periodical treatment of wells with lime.
- (c) Stocking step-wells with fish such as *Gambusia*, *Barbus*, *Haplochilus*, etc. In this respect *Saccobranchus fossilis*, *Anabas scandens*, *Clarius batrachus* and *Trichogaster (Kõlisa) fasciatus* will be found extremely useful not only for this purpose but also for controlling *Anopheles* larvæ breeding in such water.
- (d) Boiling the water for drinking purposes.
- (e) Straining drinking water through a piece of thick muslin in order to hold back cyclops including their young ones.

Genus *Daphnia*.

These live in stagnant pools and may be mistaken for cyclops from which they differ in the body being unsegmented and lying within a bivalve shell from which the beak-like head and the antennæ project. *Daphnia* do not take any part in the spread of guinea-worm disease.

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MISCELLANEOUS ORDERS OF INSECTS

There are a large number of insects which are of considerable economic significance to us. In many of them man is seldom concerned with their adult life

but in their larval stages they generally act as natural enemies of larvæ of mosquitoes and of other aquatic insects. Some of these orders are, Odonata (Dragon-flies), Ephemera (May-flies), Plecoptera (Stone-flies), Neuroptera (Alder-flies) and Trichoptera (Caddis-flies). Their larvæ are found in ponds, ditches,

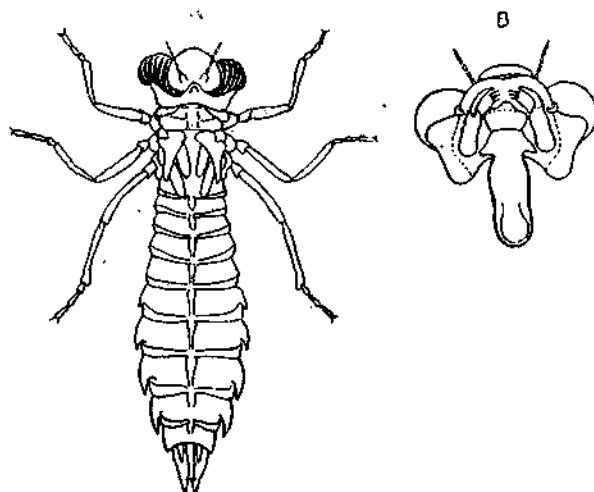


Fig. 158

A. Larva of *Aeschna* (Dragon-fly). (After Miall).

B. Mouth parts of larva of *Aeschna* (After Miall).

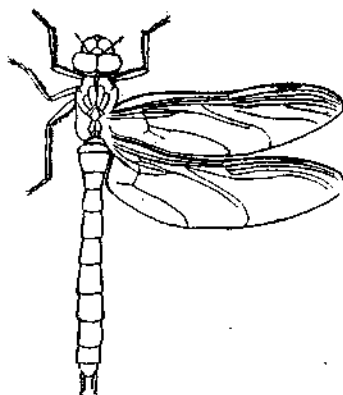


Fig. 159

Dragon Fly (Odonata).

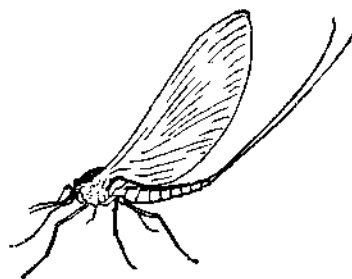


Fig. 160

Ephemerid.

pools, etc. They are carnivorous and feed on other aquatic insect larvæ. The mouth parts are composed of well developed mandibles. It may be pointed out that the application of mineral oil will kill not only mosquito larvæ but also larvæ of these somewhat useful insects.

NOTES ON ENTOMOLOGICAL TECHNIQUE

How to preserve insects in the laboratory.

- (1) They are mounted on suitable-sized pins or preserved in store boxes.
- (2) They may be preserved in dry glass tubes with tight-fitting corks. A few fragments of naphthalene are placed at the bottom of the tube, and prevented from being shaken about by cotton wool placed above them.

How to preserve unmounted adult insects of small size and their larvæ.

Any of the following preservatives may be used.

- (1) 4 per cent formaldehyde solution.
- (2) 70 per cent spirit.

Borax	gr. 5
Glycerin	2.5 cc.
Formaldehyde (40 per cent.)	100 cc.
Distilled water add to make	1 litre.
- (4) Bles's fluid.

Alcohol (70 per cent.)	90 cc.
Formaldehyde	7 cc.
Glacial acetic acid	3 cc.
- (5) Alcohol (95 per cent.) 80 cc.

Formaldehyde (10 per cent.)	15 cc.
Glycerin	5 cc.

How to kill adult insects.

1. Insects may be allowed to die in test-tubes. The only disadvantage is that in the case of mosquitoes, the longer these insects live, the greater are the chances of the legs and wings being damaged.

2. Chloroform vapour may also be used for killing insects. When insects die slowly, the legs and wings remain fully stretched which is the ideal state for mounting insects on pins. It is therefore desired that the minutest amount of chloroform vapour necessary to stupefy them should be used. The chloroform should never be poured on cotton-wool of the test-tube but should always be introduced inside the tubes in the form of vapour by means of a pipette.

3. Killing bottles containing cyanide are most commonly used. They are cheap and last for a long time provided they are kept in a dry atmosphere. To make such bottles or tubes place some cyanide of potassium and sodium in the bottom; this is fully covered with a layer of dry plaster of Paris, over which is poured liquid plaster of Paris which is allowed to set. This is covered by several layers of filter paper and the bottle or the tube is kept tightly corked. The bottle intended to be used for this purpose must have a very wide mouth and the cork must fit tightly.

Killing and preserving mosquito larvae.

(1) Mosquito larvæ may be killed and preserved at the same time in 70 or 80 per cent spirit or 4 per cent formaldehyde. Formalin has a tendency to harden the object and spirit appears to be a more satisfactory preservative.

(2) Mosquito larvæ may be transferred from water to freshly or recently prepared Bles's fluid. After 24 to 48 hours the objects are transferred to ordinary 70 per cent spirit.

(3) All Dipterous larvæ are readily killed when dropped in water heated to 80°C. They may thereafter be preserved in either 70 per cent spirit or in any other preserving fluid. Mosquito larvæ retain all their fine hairs when killed in this way.

How to mount mosquitoes on a pin.

Place the mosquito on its back on a piece of cork ; for mosquitoes use No. 10 (nickel coated) pins. Hold the head of the pin with a pair of forceps, pass the pointed end through a piece of pith and then through the thorax of the mosquito taking particular care that the end does not pass through any of the joints of the legs and the sharp end is just visible on the dorsum of the thorax. An ordinary office pin is then passed through the other end of the pith, and the mosquito preserved in the store box.

Store Boxes.

Store boxes for storing pinned specimens must be light and are generally made of teak or wood. They should be of a standard size and the lid must be well fitting. A cork carpet is cut to fit the floor. The cork may be fixed to the floor by a few small pins or by means of paraffin wax. A mixture of paraffin (melting point 163°F.) 80 per cent and flake naphthalene 20 per cent is melted and a small quantity is run into the box ; the cork is at once put in and this is held down by the paraffin ; more of the mixture is then run in, sufficient to cover the cork completely. The box is ready for use when cold.

As a preventive against mould and insects like mites and ants, creosote mixed with chloroform is commonly used. This should be poured on some cotton wool wound round the head of a pin, the latter being fixed to the cork. Too much of the chloroform and creosote mixture is likely to cause damage to specimens by the condensation of creosote on the wings and body of the insects.

Ordinary cigar boxes will make good store boxes for field work.

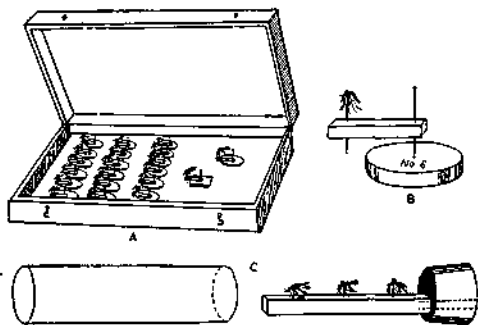


Fig. 161

A, Box for storing pinned insects. B, A mosquito mounted on a pin. C, Easy method of keeping a large number of mosquitoes in a tube. In this way preserved insects can be despatched by post.

How to mount entomological objects permanently on glass slides.

A large number of methods have been advocated but the most commonly used clearing agents are carbolic acid, lactophenol and gum-chloral mixture.

Carbolic acid method.

(a) Transfer the object to a watch glass ; in the case of aquatic insects, the water must be drained off.

(b) Pour sufficient liquid carbolic acid to drown the object.

(c) As soon as the clearing and dehydration is complete, *i.e.*, when the object looks quite transparent (this takes from 10 minutes to half-an-hour or more), the carbolic acid is drained off and a few drops of oil of clove is put in its place.

(d) After about 5 to 10 minutes, the object is carefully transferred to a slide in a drop of clove oil, care being taken not to injure the object in any way. Mounted needles and camel hair brushes should be used for this purpose.

(e) With the help of needles the object is properly oriented under a dissecting microscope. Extra clove oil is now wiped off with filter paper and the object is mounted in the usual way in a thin drop of canada balsam dissolved in xylol. It is covered with a cover-glass.

Other clearing and mounting media.

A. Aman's lactophenol.

Phenol crystals	1 gm. or 20 c.c.
Lactic acid	1 gm. or 20 c.c.
Glycerin	2 gm. or 40 c.c.
Water	1 gm. or 20 c.c.

B. Mukerji's lacto-chloral.

Chloral hydrate	0.5 gm.
Distilled water	1 cc.
Glycerin	1 cc.
Lactic acid	2 cc.
Glacial acetic acid	2.4 minims.
Formal	0.5 cc.

Both are good clearing and mounting media. The margins of the cover-glass should be ringed with melted wax or some enamel paint.

C. Berles's fluid.

Chloral hydrate	160 gm.
Gum arabic	15 gm.
Glucose	10 gm.
Glacial acetic acid	5 c.c.
Water	20 cc.

It is not only a good clearing but also a good mounting medium especially for small-sized objects and is therefore extensively used in entomological work. In using gum-chloral mixture insects preserved in 70 per cent alcohol should be washed in water, then treated with 10 per cent glacial acetic acid and finally mounted in

gum-chloral mixture. The edge of the cover-glass should always be ringed with wax. It takes about 2 to 3 days for the gum to harden.

D. Gater's fluid.

Distilled water	10 cc.
Gum arabic	8 gm.
Chloral hydrate	74 gm.
Glucose syrup or glycerin	5 cc.

The ingredients should be dissolved in the order named preferably on a water bath at 50°C. The fluid should be filtered by means of a Buchner funnel and suction pump. Glucose syrup is made by dissolving 98 gm. glucose in 10 ml. of water.

Hetherington's solution.

Absolute alcohol	20 cc.
Chloroform	15 cc.
Acetic acid (glacial)	5 cc.
Phenol crystals	10 cc.

Advised for killing, fixing, clearing and dehydrating Diptera larvæ and non-pigmented insects.

When specimens appear clear enough they are transferred to a mixture of this solution and oil of wintergreen in equal parts for 15 minutes, thereafter to pure oil of wintergreen for 12 hours. They are mounted in neutral balsam containing a few drops of oil of wintergreen.

Transporting adult mosquitoes and mosquito larvæ by post.

A. Living mosquitoes.

For transporting living mosquitoes by post under all conditions Barraud's cage is very suitable.

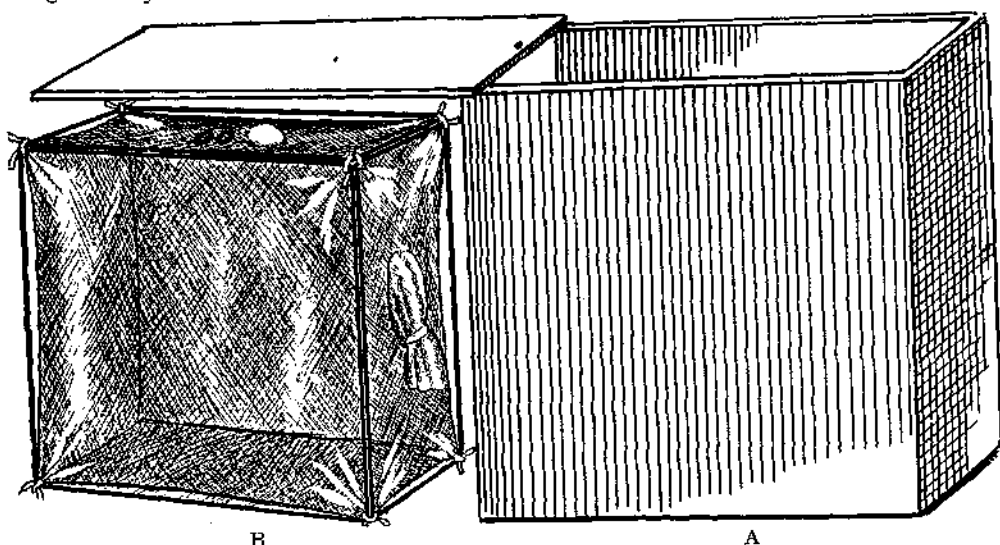


Fig. 162

Barraud's box (A) with sliding lid in which is placed Barraud's cage (B) containing living mosquitoes for transportation by post or railway.

A framework is constructed by tying firmly together at the corners with tapes six squares of strong wire measuring about 6 inches along each side. On the inside of this frame a cage of small-mesh mosquito netting is fastened by means of tape sewn to the corners of the net, to one side of which a small sleeve is attached large enough to admit a test-tube. The cage is placed in a wooden box. The mosquitoes are introduced into the cage, through the sleeve, which is then secured with tape. A folded pad of wet lint is placed at the bottom of the box, the pad being sufficiently thick to press against the bottom of the mosquito netting. For the nourishment of the mosquitoes some raisins are deposited on the top of the cage and kept in place by a thick pad of cotton-wool.

B. Dead mosquitoes.

(a) The mosquitoes are placed in a dry tube, there being only one specimen between two layers of wool so that all remain separate. A preservative in the form of naphthalene or a drop of creosote should be used.

(b) The mosquitoes after being mounted on pins, are arranged side by side on a flat piece of cork fixed by pins to the cork of the glass tube inside which the mosquitoes are preserved.

C. Mosquito larvæ.

They are packed in a corked tube, half of which is filled with spirit and the rest filled with small strips of paper; this will prevent the objects from being shaken during transit.

Sticking fluid.

For sticking damaged insects ordinary gum is useless. Celluloid chopped up in amyl acetate makes a sticky mixture and this is generally used for sticking legs and wings of damaged insects.

Preparation of different strengths of alcohol from rectified spirit.

The lower strength of alcohol should always be made from rectified spirit which contains 96 per cent of alcohol. The quantity of water and spirit to be used in order to prepare a certain strength is shewn below:—

Rect. spt. parts.	Water parts.	Required strength %
2	74	25
31	69	30
41.5	58.5	40
52	48	50
62.5	37.5	60
73	27	70
78	22	75
83	17	80
93.5	6.5	90

Absolute alcohol.

It is more advisable to prepare absolute alcohol from rectified spirit by adding dehydrated CuSO_4 to the latter. The last trace of water that will still be left in specimens treated with this alcohol can be easily removed with xylol and clove oil.

Section Cutting.

Section cutting includes three different operations, *e.g.*, (1) fixation in any of the many well-known fixative agents ; (2) clearing or dehydration in ascending grades of alcohol and (3) embedding in paraffin (single embedding) or in celloidin first and then in paraffin (double embedding).

Fixation and dehydration.

For general insect work two fixatives are commonly used. These are (1) Bouin's fluid and (2) Bles's fluid.

Bouin's fluid. "

Saturated solution of picric acid in water	...	75 cc.
Formaldehyde, 40 per cent	25 cc.
Acetic acid	5 cc.

Bles's fluid.

Alcohol, 70 per cent	7 cc.
Formaldehyde	7 cc.
Glacial acetic acid	3 cc.

Bles's fluid may be used either cold or hot. The object may be left in either Bles's or Bouin's fluid for 24 to 48 hours. As far as possible living insects are dropped in the fixative and after 6 to 12 hours one or two nicks are made in its body to allow the fixative to penetrate into every part of the insect. The object is generally transferred from Bouin's fluid to 30 per cent and from Bles's fluid to 70 per cent alcohol. Thereafter dehydration is effected in ascending grades of alcohol, *e.g.*, 30, 50, 70, 80, 90, 96 per cent and last of all in absolute alcohol. Up to 80 per cent alcohol a mosquito or any other small object does not generally require treatment for more than 6 hours but in each of the higher strengths the object should be kept for 12 hours. Two changes of absolute alcohol are desired. From absolute alcohol pass the object through absolute alcohol + xylol in equal parts for 1 hour, xylol for $\frac{1}{2}$ hour when it is ready for embedding.

Hennig's solution (particularly recommended for mosquitoes and sandflies).

Nitric acid	16 parts.
Chromic acid 0.5 per cent	16 parts.
Saturated solution of mercuric chloride in 60 per cent alcohol	24 parts.
Saturated solution of picric acid in water	12 parts.

Fix for one day ; then put in a mixture of Lugol's solution and 70 per cent alcohol for 2 hours. Pass through two changes of 70 per cent alcohol till no trace of iodine remains.

If necessary the whole object may be stained in Ehrlich's hæmatoxylin for 3 days after fixation in Hennig's solution.

The subsequent process of dehydration is carried out in the usual way.

Embedding.

Double embedding in celloidin and paraffin: On account of the chitinous exoskeleton of insects, double embedding with celloidin and paraffin will yield more satisfactory results than single embedding with paraffin alone. For double

embedding, a thick and a thin solution of celloidin dissolved in clove oil are required. The thick solution is first prepared. It has the consistency of thick treacle. This is diluted with clove oil to make a very thin solution. It must be remembered that celloidin dissolves in clove oil slowly and it takes several days to prepare the stock solutions.

The object is transferred from xylol to thin solution of celloidin and left for 2 days and thereafter transferred to the thick solution for 2 days.

Now take a cover glass and dip it in melted paraffin. Take a large drop of thick celloidin solution on the paraffin-coated cover glass and wait till all air bubbles have disappeared. Quickly transfer the object to the thick celloidin on the cover glass taking particular care that in doing this no new air bubbles are introduced. The object will gradually sink in the celloidin solution and if necessary another drop of celloidin may be poured on the object in order to cover it completely. Lift the cover slip with forceps and turn it over so that the drop hangs down with the object in it. Quickly lower it into chloroform in a corked tube. The celloidin will soon harden into a jelly and as soon as the paraffin is dissolved, the object is detached from the cover glass.

The object is now trimmed down so as to leave a moderate thickness all round it. It is now put in a tube containing chloroform into which shreds of paraffin wax with a melting point 58°F are gradually dropped till the mixture is saturated with paraffin wax. Leave the object in the paraffin-chloroform mixture for 24 hours when the object will sink at the bottom. It is now ready for paraffin embedding.

Paraffin embedding should be done in an incubator at a constant temperature of $58\text{--}60^{\circ}\text{F}$. The object is transferred from the chloroform mixture to paraffin and thereafter to two subsequent changes of paraffin. After this the block is made in a paper capsule or in a watch glass smeared with glycerine. As soon as the paraffin begins to harden, the watch glass is gradually plunged in water beginning from its edge.

Sections are cut generally 7 to 10μ thick with a microtome.
Section staining.

The sections are arranged on a glass slide in water, the slides having been just previous to this carefully smeared with a minute drop of egg albumen. Gentle heat applied to the water will cause the sections to stretch. The slide is now put away for one or two days till they are completely dry.

For staining, pass the sections through (1) Xylol. (2) Absolute alcohol. (3) Descending grades of alcohol. (4) Distilled water. (5) Delafield's or Ehrlich's hæmatoxylin for 2 to 5 minutes. (6) Rinse in water; blueing in tap water for a few hours is essential. (7) If overstained, dip in a jar of weak solution of HCl (0.5%) for 5 to 30 seconds. (8) Rinse quickly in water. (9) Blue again in tap water. (10) 4 per cent aqueous solution of eosin for 1 minute. (11) Rinse rapidly in water. (12) Dehydrate in ascending grades of alcohol till absolute alcohol is reached. (13) Absolute alcohol+xylol. (14) Xylol. (15) Clove oil. (16) Mount in a drop of Canada balsam and cover with a cover glass.

After treatment with hæmatoxylin and blueing in water, the object may be passed through ascending grades of alcohol till 70 per cent spirit is reached. At

this stage any overstaining with hæmatoxylin is corrected by 1 per cent solution of picric acid in alcohol but the object must not be left for more than half to one minute. Then treat with 70 per cent and 90 per cent alcohol. It is now stained with 2 per cent eosin solution prepared in 90 per cent alcohol for 1 minute. Dehydration is now effected with 95% alcohol, absolute alcohol, xylol, and clove oil. After this the sections are mounted in a drop of Canada balsam in the usual way. Staining whole objects.

The entire object when small may be stained either in Ehrlich's acid hæmatoxylin for 3 days or in Mayer's hæmalum or acid hæmalum for one to three days. The object may either be mounted whole or may be used for section cutting. Mayer's acid hæmalum is extremely useful for demonstrating embryos of guinea-worm ~~undergoing~~ development in cyclops. Mosquitoes, sandflies, etc., may be first stained either with hæmatoxylin or hæmalum and then mounted on slides.

Ehrlich's acid hæmatoxylin.

Hæmatoxylin	...	2 gm.	Dissolve hæmatoxylin in alcohol and then add the rest. Ripen it by exposing it to light and oxygen. Oxygen can freely enter through the cotton-wool plug.
Absolute alcohol	...	100 cc.	
Glycerin	...	100 cc.	
Distilled water	...	100 cc.	
Glacial acetic acid	...	10 cc.	
Potash alum in excess.			

After treatment with acid hæmatoxylin leave the object in tap water for a few hours for blueing.

Mayer's hæmalum.

Hæmatin	1 gm.
Alcohol, 90 per cent	50 cc.
Pot. alum.	50 gm.
Aqua dist.	100 cc.

Mayer's acid hæmalum.

Add 20 cc. of glacial acetic acid to the above when objects are overstained; differentiate in 0.5 per cent HCl. in distilled water. Immerse in ordinary tap water for 2 hours (add a few drops of strong ammonia). Dehydrate, clear in ascending grades of alcohol and mount in balsam.

DeLafield's hæmatoxylin.

Prepare 400 cc. of a saturated solution of ammonia alum (1 part of alum to 11 parts of distilled water).

Dissolve 4 gm. of hæmatoxylin in 25 cc. of absolute alcohol in a flask with cotton-wool plug and expose it to light for about a week. Filter. Add to the filtrate 100 cc. of glacial acetic acid and 100 cc. methylic alcohol. Leave in a warm place for six weeks.

DeLafield's stain acts much more quickly than Ehrlich's hæmatoxylin.

Frozen sections.

Frozen sections of 7 to 10 μ in thickness are sometimes necessary for the demonstration of fat cells. Such sections cannot be mounted permanently. These are temporarily mounted in a drop of glycerin.

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